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MORPHOLOGICAL DIFFERENCES BETWEEN THE "RACES" OF DROSOPHILA PSEUDOOBSCURA

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1. INTRODUCTION

THE two "races" of *Drosophila pseudoobscura,* known as "race A" and "race B," respectively, were first distinguished by Lancefield (1929), who found that on intercrossing they produce sterile male hybrids. In addition to this sterility and other abnormalities of the hybrids, Lancefield further found an incomplete sexual isolation of the races: in mixed cultures intra-racial matings were more common than inter-racial matings.

The only visible difference between the two races detected by Lancefield was that in the shape of the Y chromosome, which was V-shaped in race B and J-shaped in race A. Dobzhansky and Boche (1933) and Dobzhansky (1935a, 1937a) have shown, however, that the shape of the Y chromosome is variable in both races, five distinct forms being present in race A and three in race B. Further, as far as can be determined cytologically, some strains of each race have the same type of Y chromosome. Tan (1935) and Koller (1936) have found the chromosomes of the two races to differ in four inverted sections, two of which lie in the X and one in each of the second and third chromosomes. Again it was shown later that the chromosome structure of the individual races was vari-

able. Certain strains of the two races are identical with respect to gene arrangement in the right limb of the X and in the third chromosomes (Sturtevant and Dobzhansky, 1936; Dobzhansky and Sturtevant, 1938). Only the left limb of the X and the second chromosome may be used as racial differences.

There can be no doubt that the two races represent distinct ecological types. Their geographical distributions are different, and in the regions where they occur together race A occupies warmer and drier habitats than race B (Dobzhansky, 1937b). Poulson (1934) has established that the developmental period of race B is longer than that of race A; the difference being mainly in the length of the pupal stage. The number of eggs deposited by a female of race A is greater than that deposited by one of race B at higher temperatures, while the reverse is true for lower temperatures (Dobzhansky, 1935b). The egg-laying curves of the races differ at all temperatures that have been tried. There is also some reason to believe that the oxygen consumption per unit dry weight differs in the two races (Dobzhansky and Poulson, 1935). Finally, in the absence of food, individuals of race A survive longer than do those of race B (Lilleland, 1938).

In short, the differences between the races are sufficiently profound to remove any hesitation to regard them as species if it were not for the apparent absence of any external morphological differences. The situation confronting us here is not entirely unique in biology, since the existence of types which are physiologically distinct and yet seem morphologically identical is known elsewhere. Such types are often designated by the not too fortunate terms of "biological species" and "biological races." It would seem, however, desirable to have further information about *Drosophila pseudoobscura,* as it is the best studied case of this type.

The genitalia, which often serve to differentiate species of insects, were carefully compared in the two races, but were found to be identical. Professor G. F. Ferris, who

kindly consented to check these observations, reached the same conclusion (private communication). The only hopeful indication of morphological difference found up to the present was obtained in measuring the length of the hind tibiae and counting the teeth of the sex combs in five strains of race A and three of race B (Dobzhansky, 1935c). It remains, however, to be ascertained whether these differences are truly interracial. Each strain consists of the descendants of a single trapped female, fertilized in the wild by one or more males, and kept in the laboratory, in some cases, for many generations. The differences between such strains of the same race could be mistaken, without careful analysis, for genuine racial differences. In order to investigate further this possibility and to establish the racial difference, if it exists, the present work was undertaken.

2. Material and Methods

Nineteen strains of race A and twenty of race B were used in the experiments. These strains were obtained from different parts of the distribution area of the species. The strains and their places of origin are given in Table 1.

TABLE 1

Race A	Race B
Mara (Shuswap) Lake–3. British Columbia*	Quesnel–5. British Columbia*
Olympic–2. Washington*	150 Mile House–5. British Columbia§
Crater Lake–3. Oregon*	Pavilion–6. British Columbia§
Black Hills–5. South Dakota*	Merritt–4. British Columbia*
Pike's Peak–4. Colorado§*	Campbell River–3. Vancouver Island§*
Tree Line–3. Colorado§	Cowichan–6. Vancouver Island§
Julian E–6. California*	Quinault–23. Washington§
Sequoia–15. California§	Quilcene–4. Washington*
Wawona–6. California§	Seattle–4. Washington§
Lida–31. California§	The Dalles–7. Washington§
Coso–99. California§	Reedsport–2. Oregon§*
Awavaz–4. California§	Sisters–9. Oregon*
Kingston–1. California§	Crater Lake–2. Oregon§
Santa Catalina–2. Arizona§	Lassen–2. California*
Santa Rita–1. Arizona§*	Lassen–8. California§
Zuni–8. New Mexico*	Dunsmuir–8. California§
Georgetown. Texas§	Wawona–4. California*
Cuernavaca–8. Mexico*	Sequoia–5. California§
Oaxaca–4. Mexico*	Sequoia–14. California*
	Nojogui–8. California*

* Wings and tibiae measured and teeth of proximal sex comb counted.
§ Teeth of proximal and distal sex combs counted.

Approximately 20 (or less) females and an equal number of males, of each strain, were placed in a culture bottle

at 24.5° C., and were transferred to fresh bottles daily. The cultures with eggs in them were allowed to develop at 24.5° C. except where otherwise stated. Cultures that proved to be overpopulated with larvae were then discarded. In the remaining bottles the flies emerging in the first three days were removed and preserved in alcohol. In this way each sample of flies from which measurements were taken comprised individuals from a number of different bottles, though all from the same parents. This technique should on the one hand insure that the flies have developed under good conditions and on the other reduce that accidental variation due to fluctuating environmental conditions in different bottles. The suitability of the method will be discussed later in the light of the experimental results.

3. RESULTS

(*a*) *Sex Combs.* Five characters were studied in the experimental material, three of which, wing length and breadth and tibia length, could be measured on flies of both sexes. The other two, *viz.*, the numbers of teeth in the proximal and distal sex combs of the right front leg, were only to be obtained from males. Two experiments were performed on two different occasions, the proximal sex combs being followed at both times, whereas the wing and leg measurements were only taken on one of the two occasions and the distal sex combs counted in the other set of material. The different strains used in each of the two experiments are marked by the symbols § and * in Table 1. As four of the strains were followed in both experiments we have twice as many observations on the proximal sex combs of these strains as of the others. The other measurements were followed in but one of the two experiments, and so an equal number of flies from each of the particular strains used were recorded. We may consider the sex comb results first.

As indicated in the introductory section we are concerned with analysis at three levels, variation between individuals of the same strain, between strains of the same

race, and finally between the two races. The most suitable statistical technique for handling a problem of this kind is that of the analysis of variance.

The mean numbers of teeth ($\bar{x}_p$) in the proximal sex combs of each of the thirty-nine strains, together with the sums of squares of deviations of individuals from the strain mean denoted by $S(x_p^2)$, are given in Table 2. The results for the four strains used on both occasions comprise counts on fifty male flies, but the remaining strains included but twenty-five males each.

A simple analysis of variance may be performed on the data of Table 2. As there are four strains, each including

TABLE 2

FREQUENCY OF TEETH IN THE PROXIMAL SEX COMBS

Race A	$\bar{x}_p$	$S(x_p^2)$	Race B	$\bar{x}_p$	$S(x_p^2)$
Santa Catalina–2	6.36	5.76	Quinault–23	6.00	2.00
*Pike's Peak–4	6.00	14.00	150 Mile House–5	5.60	6.00
Kingston–1	5.92	5.84	Pavilion–6	5.64	5.76
Coso–99	6.44	8.16	The Dalles–7	5.76	6.56
*Santa Rita–1	6.22	12.58	Dunsmuir–8	5.96	8.96
Tree Line–3	6.56	8.16	Sequoia–5	5.73	5.04
Awavaz–4	6.64	5.76	Lassen–8	5.64	7.76
Texas	6.68	7.44	Crater Lake–2	5.44	6.16
Lida–31	6.72	9.04	Seattle–6	5.04	2.96
Sequoia–15	6.76	8.56	Cowichan–6	4.56	6.16
Wawona–6	6.72	5.04	*Reedsport–2	5.56	12.32
Olympic–2	6.64	9.76	*Campbell River–3	5.82	9.38
Crater Lake–3	6.76	6.56	Quilcene–4	5.76	4.36
Zuni–8	6.48	10.24	Quesnel–5	5.72	9.04
Julian E–6	6.88	6.64	Sisters–9	6.08	5.84
Cuernavaca–8	5.96	2.96	Lassen–2	5.36	5.76
Oaxaca–4	6.40	8.00	Sequoia–14	5.88	6.64
Black Hills–5	6.80	10.00	Nojogui–8	5.76	10.56
Mara–3	6.36	7.76	Wawona–4	5.92	3.84
			Merritt–4	5.20	6.00
Racial mean	6.4533		Racial mean	5.6273	

All these results are based on counts of 25 males except those marked * which are based on 50 males.

fifty individuals, and thirty-five, including twenty-five individuals, we have observations on 1,075 flies in all. There is thus a total of 1,074 degrees of freedom in the analysis. As there are thirty-nine strains, 38 degrees of freedom are concerned with racial and strain differences, leaving 1,036 for the variation of individuals round the means of their strains. The 38 degrees of freedom may be further subdivided into 1 for the difference between the two races and 37 for deviations of strains round

their racial means. The arithmetic method of obtaining the sums of squares of deviations of individuals round the strain means, of strains round the racial means, and finally between the races is fully described by Fisher (1936a and 1936b) and need not be discussed here. Having found the requisite sums of squares from the data of Table 2 we obtain the analysis of variance given in Table 3.

TABLE 3

THE ANALYSIS OF VARIANCE OF NUMBER OF PROXIMAL SEX COMB TEETH

	Sum of squares	Degrees of freedom	Mean square
Races	183.2893	1	183.2893
Strains	109.1377	37	2.9497
Individuals	283.5600	1,036	0.2737
Total	575.9870	1,074	

It is clear that there is a significant difference between strains of the same race, as in the absence of such differences the mean squares (or variances) of "strains" should be equal to the mean square of "individuals." The calculation of z ($\frac{1}{2}\log_e$ of the ratio of these variances) gives a value of 1.1887, which, for 37 degrees of freedom against 1,036, has a probability considerably less than 1 per cent. of occurring by chance. Hence in testing the evidence for the existence of a difference between the races we must compare the "race" mean square with that for "strains" and not with the value for "individuals," as otherwise we might be led into the error of ascribing to racial difference an apparently significant variance which was really a mere difference between strains. A comparison of the "race" variance with the "strain" variance renders such an error impossible. In making this comparison of the "race" and "strain" mean squares we obtain a z of 2.0647, or a t value of 7.883. Either test of significance indicates a very real difference between the races, when entered in the appropriate tables given by Fisher (1936a). Thus the number of teeth in the proximal sex comb provides a genuine external morphological difference between the two races.

The distal sex combs of 25 males from each of 23 strains, 11 of race A and 12 of race B, were also counted, in addition to the proximal comb data. In Table 4 will

TABLE 4

FREQUENCIES OF TEETH IN THE PROXIMAL AND DISTAL SEX COMBS

	$\bar{x}_p$	$\bar{x}_d$	$S(x_p^2)$	$S(x_d^2)$	$S(x_p x_d)$	$\bar{X}$
Race A:						
Santa Catalina–2	6.36	5.24	5.76	4.56	0.84	8.59
Pike's Peak–4	5.92	5.12	5.84	2.64	0.24	8.45
Kingston–1	5.92	5.36	5.84	5.76	1.72	8.57
Coso–99	6.44	5.64	8.16	5.76	2.96	9.23
Santa Rita–1	6.40	5.16	6.00	9.36	5.40	8.95
Tree Line–3	6.56	5.56	8.16	6.16	1.16	9.31
Awavaz–4	6.64	5.36	5.76	5.76	0.24	9.29
Texas	6.68	4.96	7.44	0.96	−0.32	9.13
Lida–31	6.72	5.48	9.04	6.24	1.36	9.43
Sequoia–15	6.76	5.60	8.56	6.00	1.60	9.53
Wawona–6	6.72	5.08	5.04	1.84	0.56	9.23
Racial mean	6.4655	5.3236				9.10
Race B:						
Quinault–23	6.00	4.88	2.00	2.64	0.00	8.42
150 Mile House–5	5.60	4.64	6.00	7.76	3.40	7.90
Pavilion–6	5.64	4.96	5.76	0.96	−0.36	8.09
The Dalles–7	5.76	4.80	6.56	6.00	3.80	8.14
Dunsmuir–8	5.96	5.08	8.96	5.84	0.08	8.47
Sequoia–5	5.72	5.04	5.04	0.96	0.28	8.21
Lassen–8	5.64	4.96	7.76	0.96	0.64	8.09
Crater Lake–2	5.44	4.88	6.16	4.64	2.32	7.86
Seattle–6	5.04	4.44	2.96	6.16	0.56	7.24
Cowichan–6	4.56	4.04	6.16	0.96	0.44	6.56
Reedsport–2	5.48	4.20	6.24	4.00	1.60	7.56
Campbell River–3	5.76	4.80	6.56	6.00	1.80	8.14
Racial mean	5.5500	4.7267				7.89

All results are based on 25 males.

be found the mean numbers of teeth of the two combs ($\bar{x}_p$ and $\bar{x}_d$) together with the sums of squares of deviations of individual observations on each comb round the appropriate strain means [denoted by $S(x_p^2)$ and $S(x_d^2)$] and also the sums of cross products of deviations of the individual observations [$S(x_p x_d)$]. In the case of the four strains used in both experiments, on only this one particular occasion were the distal combs counted and so but 25 males of these strains, as of the others, are recorded in Table 4. In Table 5 are the analyses of variance and co-variance, the last based on the sums of cross products, of the teeth frequencies of the two combs. There is 1 degree of freedom for racial differences, 22 − 1, *i.e.*, 21, for differences of strains within the races, and 574 − 22,

TABLE 5

ANALYSES OF VARIANCE AND CO-VARIANCE OF PROXIMAL AND DISTAL COMB TEETH

	Sum of squares	Degrees of freedom	Mean square
Variance of Proximal Teeth:			
Races	120.2430	1	120.2430
Strains	66.9118	21	3.1863
Individuals	145.7600	552	0.2641
Variance of Distal Teeth:			
Races	51.1317	1	51.1317
Strains	43.8631	21	2.0887
Individuals	101.9200	552	0.1846
Co-variance of Proximal and Distal Teeth:			
Races	78.4107	1	78.4107
Strains	32.3545	21	1.5407
Individuals	30.3200	552	0.0551

i.e., 552, for variations between individuals of the same strain. In each case the variance, or co-variance, between strains of the same race is larger than that of individuals of the same strain. The values for the racial differences are also larger than those for the differences between strains of the same race, just as before. Further, since the values for the two combs are not completely correlated ($r = +0.597$ between strain means) some information presented by each comb is not to be obtained from a comparison based solely on the other one. Thus some composite score, based on a combination of the numbers of teeth in the two combs, will be better than the values of either comb taken separately.

We may proceed to find such a score, or "Discriminant Function," by the method of Fisher (1936c). This consists of taking a linear function X of the two variables, x_p (proximal comb teeth) and x_d (distal comb teeth), such that the difference in score between the two races is maximized with respect to the variability of X within the races. As we have shown that there exist real differences between the strains of a race we must compute the variability of X within the races by the use of strain means and not by using individual observations.

We give the two variables, x_p and x_d, the coefficients λ_p and λ_d in finding the composite score X. Thus, an extra suffix denoting A or B,

$$\overline{X}_A = \lambda_p \overline{x}_{pA} + \lambda_d \overline{x}_{dA}$$

and

$$\overline{X}_B = \lambda_p \overline{x}_{pB} + \lambda_d \overline{x}_{dB}$$

giving by subtraction

$$D = \overline{X}_A - \overline{X}_B = \lambda_p d_p + \lambda_d d_d$$

where d_p is the difference between the race averages of x_p, and d_d is similarly $\overline{x}_{dA} - \overline{x}_{dB}$.

The theory and calculation of discriminants is fully described by Fisher (*loc. cit.*). We require for this purpose the sums of squares of deviations of the strain means, for both x_p and x_d, from the appropriate racial means, and also the sum of cross products of these deviations. These are obtained from the second row of the analyses of Table 5, *i.e.*, the three entries in the "strains" row. We divide these values by 25, however, as the strain means are the averages of 25 observations each, whereas the sums of squares in Table 5 are based on the values of single males.

The values of λ_p and λ_d are given by the solution of the equations

$$\begin{aligned} S(x^2_p)\lambda_p + S(x_p x_d)\ \lambda_d &= d_p \\ S(x_p x_d)\lambda_p + S(x^2_d)\ \lambda_d &= d_d \end{aligned}$$

where $S(x_p{}^2)$ is the sum of squares of x_p, $S(x_d{}^2)$ the sum of squares of x_d, and $S(x_p x_d)$ is the sum of cross products. Substituting the appropriate arithmetic values, we find

$$\begin{aligned} 2.6765\lambda_p + 1.2942\lambda_d &= 0.9155 \\ 1.2942\lambda_p + 1.7545\lambda_d &= 0.5965 \end{aligned}$$

and so

$$\lambda_p = 0.2760 \text{ and } \lambda_d = 0.1366$$

or putting λ_p equal to 1

$$\overline{X} = \overline{x}_p + 0.4949\ \overline{x}_d$$

Then D, the difference between the average scores of the two races, is

$$0.9155 + (0.4949 \times 0.5969), \textit{i.e.}, 1.2109.$$

The significance of the racial difference shown by this score may be tested by analyzing the variance of X into its two parts, between the races and between strains of the same race. It will be noticed that this test of signifi-

cance is precisely analogous to the test applied to the individual comb values.

It has been shown by Fisher (*loc. cit.*) that the sum of squares between races is given by

$$\frac{n_A n_B}{n_A + n_B} D^2$$

where n_A and n_B are the numbers of strains of the two races used in the experiment. In the present case n_A is 11 and n_B is 12. Then the sum of squares between races is

$$\frac{132}{23}(1.2109)^2$$

i.e.,

$$8.4147$$

The sum of squares between strains of the same race is found from the formula

$$\lambda^2_p S(x^2_p) + 2\lambda_p\lambda_d S(x_p x_d) + \lambda^2_d S(x^2_d)$$

and, as we have put $\lambda_p = 1$ and $\lambda_d = 0.4949$, is

$$2.6765 + [0.9898 \times 1.2942] + [(0.4949)^2 \times 1.7545]$$

or

$$4.3862.$$

There are 23 strains in all and so a total of 22 degrees of freedom. In our previous analyses of the values of each comb taken singly we have subdivided these 22 into 1 between races and 21 between strains of the same race. As we have, however, now emphasized the difference between the races by fitting an adjustable parameter, viz., the ratio of λ_p to λ_d, we must assign 2 degrees of freedom to the racial difference and leave but 20 for the strain variation instead of the original 21. The analysis of variance of X, obtained in this way, is given in Table 6.

It is desirable to test the increase in accuracy of detection of the racial difference by the X score over that obtained when either comb is used separately. This can not be done directly, as the X score has 2 degrees of freedom against 20, whereas the single comb data have 1 against 21. We may, however, achieve this purpose indirectly by

TABLE 6

ANALYSIS OF VARIANCE OF THE COMPOUND SCORE *X*

	Sum of squares	Degree of freedom*	Mean square
Races	8.4147	2	4.2074
Strains	4.3862	20	0.2193

* z for the difference between the variances is 1.4771, which for 2 degrees of freedom against 20 is well outside the 1 per cent value of 0.8831. Thus the difference between the races as measured by our discriminant function is large and very significant.

a comparison of the frequency of misclassification. From the mean square, or variance, of strains within races we may calculate, by taking the square root, a standard error to which the strain means are subject. Before doing this, however, we must divide the variances of Table 5 by 25 just as in the calculation of the discriminant, and for the same reason, *viz.*, that the strain means are based on 25 individuals. In the case of the analysis of variance of the *X* score, as given in Table 6, the division by 25 has already been performed.

Thus the mean number of teeth of the proximal comb has a standard error of $\sqrt{\frac{3.1863}{25}}$ *i.e.*, 0.3596. That of the distal comb has similarly the standard error 0.2890 and the *X* score mean, having a variance of 0.2193, has a standard error of 0.4683.

Now misclassification will occur when a strain mean deviates from its racial mean by at least half the difference between the means of the two races, provided that the deviation is in the right direction, *viz.*, that of the other strain. The probability of finding a deviation as large or larger than this minimum difference can be found by dividing half the difference between the racial means by the standard error of the strain mean. This quotient is entered in a Table of Normal Deviates, such as is given by Fisher (1936a) or Mather (1938) and the probability of obtaining an equally large chance deviation is then found. We should note, however, that as deviations in but one of the two possible directions will lead to misclassification we must divide the probability given by the Table of Nor-

mal Deviates by two in order to obtain the probability we require.

The three quantities, proximal comb mean, distal comb mean and X score mean are analyzed in this way in Table 7.

TABLE 7

MISCLASSIFICATION OF STRAINS

	Means		Difference	Standard error	Ratio
	Race A	Race B			
Proximal comb	6.4655	5.5500	0.9155	0.3569	1.283
Distal "	5.3236	4.7267	0.5969	0.2890	1.033
X score	9.1001	7.8892	1.2109	0.4683	1.293

The probability of obtaining by chance deviations as large or larger than the one under consideration decreases as the ratio of the particular deviation to the standard error increases. Then it is clear that both the X score and the proximal comb are better for classification of races A and B than is the distal comb. Further, the X score enjoys a small but definite advantage over the proximal comb. It would indeed be surprising if this were not the case, as λ_p and λ_d were chosen to maximize the racial difference.

The Table of Normal Deviates gives the probability corresponding to a ratio of 1.293 as just less than 0.20. Dividing this by 2 we obtain as the frequency of misclassification a value just under 0.10. For various reasons discussed by Fisher, this is a maximum value for misclassification, but in our example there is no reason to expect that the real frequency will be much less than this calculated maximum.

Testing the frequency of misclassification empirically, by the calculation of the X score for each of the 23 strains of Table 4, we find that of the B strains none fall above the mid value of 8.495 and of the A strains only one falls below this value. Thus the actual misclassification in the strains used would be 0.043, a value somewhat below the expected frequency of 0.10. As only 23 strains were used, there is no reason to suspect a real difference between observed and expected misclassification.

(*b*) *Wing and Leg Measurements.* In each of the 20 strains, 10 of each race, marked with an asterisk in Table 1, the length and width of the right wing (x_l and x_w) and the length of the right tibia (x_t) was measured on each of 25 males and 25 females. The number of teeth in the proximal sex combs of the males was also recorded, as noted above. Table 8 gives the means of these measurements for each strain. Each unit is approximately 39 μ. In Table 9 will be found the sums of squares and cross products of deviations of the strain means of the various measurements from the racial means. These correspond to the sums of squares and cross products used in obtaining λ_p and λ_d in the previous section.

TABLE 8

WING AND LEG MEASUREMENTS

	Females			Males			
	$\bar{x}_l$	$\bar{x}_w$	$\bar{x}_t$	$\bar{x}_l$	$\bar{x}_w$	$\bar{x}_t$	$\bar{x}_p$
Race A :							
Olympic–2	48.14	28.70	18.94	42.02	24.86	17.46	6.64
Crater Lake–3	44.72	25.92	17.56	41.64	24.32	17.20	6.76
Zuni–8	45.86	26.60	17.20	44.06	23.98	16.22	6.48
Santa Rita–1	43.16	24.62	16.28	39.16	22.26	15.60	6.04
Julian I–6	44.88	26.30	17.38	41.10	23.90	16.90	6.88
Cuernavaca–8	44.72	24.96	16.20	41.56	23.06	15.86	5.96
Pike's Peak–4	43.90	25.32	16.56	41.88	24.40	16.74	6.08
Oaxaca–4	46.76	26.64	18.48	41.68	24.02	17.12	6.40
Black Hills–5	40.86	23.66	16.10	37.12	21.32	15.34	6.80
Mara–3	45.84	26.02	16.68	39.90	22.04	15.30	6.36
Racial mean	44.884	25.874	17.138	41.012	23.416	16.376	6.440
Race B :							
Reedsport–2	43.38	26.24	17.10	39.52	23.90	15.94	5.64
Campbell River–3 ..	44.90	25.88	16.66	39.08	22.02	14.94	5.88
Quilcene–4	45.28	26.68	17.10	38.84	22.60	15.24	5.76
Quesnel–5	46.54	27.24	17.46	42.00	24.64	16.14	5.72
Sisters–9	45.34	27.04	17.48	41.64	25.06	16.32	6.08
Lassen–2	44.08	25.32	16.80	39.50	22.80	15.80	5.36
Sequoia–14	45.04	25.72	16.92	40.36	22.52	15.92	5.88
Nojogui–8	43.68	24.36	16.68	39.44	22.22	15.86	5.76
Wawona–4	46.24	26.52	17.44	41.58	23.36	15.84	5.92
Merritt–4	46.56	26.36	17.02	41.82	23.38	15.86	5.20
Racial mean	45.104	26.136	17.066	40.378	23.250	15.786	5.720

All means based on 25 individuals.

It will be noticed that whereas the wing length and width are highly correlated both with one another and with tibia length, none of these three measurements is correlated with the number of teeth in the proximal sex comb. Hence we may inquire whether the wing and leg measurements contribute any information as to racial difference and if

TABLE 9
SUMS OF SQUARES AND CROSS PRODUCTS OF WING AND LEG MEASUREMENTS

	Wing length	Wing width	Tibia length	Sex comb teeth
Females:				
Wing length	47.8276	29.1644	16.1991	.....
Wing width	29.1644	23.5206	12.8045	.....
Tibia length	16.1991	12.8045	9.4028	.....
Males:				
Wing length	46.2078	24.9475	11.4402	−0.5904
Wing width	24.9475	21.9288	11.0084	0.7592
Tibia length	11.4402	11.0084	7.3766	0.8368
Sex comb teeth	−0.5904	0.7592	0.8368	1.6256

so whether they may be profitably combined with the sex comb values for this purpose.

Adopting the same statistical technique as before we find the coefficients λ_l, λ_w and λ_t which will maximize the difference between the females of the two races when used in the function

$$\overline{X} = \lambda_l \overline{x}_l + \lambda_w \overline{x}_w + \lambda_t \overline{x}_t$$

The racial differences in these measurements, from Table 8, and the sums of squares and cross products from Table 9, give the following as the equations of estimation of these coefficients:

$$\begin{aligned} 47.8276\lambda_l + 29.1644\lambda_w + 16.1991\lambda_t &= -0.220 \\ 29.1644\lambda_l + 23.5206\lambda_w + 12.8045\lambda_t &= -0.262 \\ 16.1991\lambda_l + 12.8045\lambda_w + 9.4028\lambda_t &= 0.072 \end{aligned}$$

Then

$$\lambda_l = 0.00658, \quad \lambda_w = -0.06686, \quad \lambda_t = 0.08738$$

and

$$D = 0.02236$$

where D is the mean difference in score between the races.

The analysis of variance between and within races may be simply performed by putting the sum of squares within races equal to 1, and the sum of squares between races equal to $\frac{n_A n_B}{n_A + n_B} D$, *i.e.*, 5×0.022362.

We note that there are 19 degrees of freedom in all and that 3 will be taken up by the inter-racial difference because we have fitted two adjustable parameters to the data. The analysis is then:

	Sum of squares	Degrees of freedom	Mean square
Races	0.1118	3	0.0373
Strains	1.000	16	0.0625

and the racial difference is clearly not significant.

A similar calculation for the wing and leg measurements of the males gives:

$$\begin{aligned} 46.2078\lambda_l + 24.9475\lambda_w + 11.4402\lambda_t &= 0.634 \\ 24.9475\lambda_l + 21.9288\lambda_w + 11.0084\lambda_t &= 0.166 \\ 11.4402\lambda_l + 11.0084\lambda_w + 7.3766\lambda_t &= 0.590 \end{aligned}$$

Then

$$\lambda_l = 0.0432, \quad \lambda_w = -0.1917, \quad \lambda_t = 0.2991$$

and

$$D = 0.1720$$

The analysis of variance is then:

	Sums of squares	Degree of freedom	Mean square
Races	0.8601	3	0.2867
Strains	1.0000	16	0.0625

and $z = 0.7617$.

This is almost equal to the value of z at the 1 per cent. point and so indicates a significant racial difference.

Thus even though these measurements detect no racial difference in the females, they serve to detect a difference when males are used.

We may then profitably combine these measurements with the counts of teeth in the proximal sex combs. The equations for the estimation of the D are from Tables 8 and 9.

$$\begin{aligned} 46.2078\lambda_l + 24.9475\lambda_w + 11.4402\lambda_t - 0.5904\lambda_p &= 0.634 \\ 24.9475\lambda_l + 21.9288\lambda_w + 11.0084\lambda_t + 0.7592\lambda_p &= 0.166 \\ 11.4402\lambda_l + 11.0084\lambda_w + 7.3766\lambda_t + 0.8368\lambda_p &= 0.590 \\ -0.5904\lambda_l + 0.7592\lambda_w + 0.8368\lambda_t + 1.6256\lambda_p &= 0.720 \end{aligned}$$

giving

$$\lambda_l = 0.0746, \quad \lambda_w = -0.1962, \quad \lambda_t = 0.2053, \quad \lambda_p = 0.4560$$

and

$$D = 0.4642$$

Now as we have fitted an extra parameter in obtaining this discriminant we have 4 degrees of freedom between the races, leaving but 15 between strains within the races. The analysis of variance is thus:

	Sums of squares	Degrees of freedom	Mean square
Races	2.3209	4	0.5802
Strains	1.0000	15	0.0667

z is 1.0815, which indicates a significant difference as its value at the 1 per cent. point is 0.7939.

We may test the increase in efficacy of classification when using this score over that obtained when the proximal comb values are used alone, by calculating the frequency of misclassification in the same way as before. The sex comb misclassification must now be calculated from the 20 strains used in these experiments and not from the 23 used in the previous section in order that it shall be comparable with the results from using the combined comb, leg and wing measurements. Then by the method of the previous section we find:

	Difference	Standard error	Ratio
Sex comb	0.720	0.293	1.229
Score	0.464	0.176	1.318

The use of the extra measurements has increased the precision of our classification and the increase is greater than that obtained by the use of the distal combs in addition to the proximal comb (see above). Thus of the measurements taken in these experiments the most accurate classification, or, alternatively, the most significant difference between the races, is obtained when using the proximal comb, the wing length and width and the tibia length. As, however, these last three measurements are more difficult to obtain, and, in any case, less accurate, it may be felt that in the majority of cases the slight increase in accuracy obtainable by their aid does not justify the extra difficulty of taking them. The numbers of teeth in the proximal and distal combs are very easy to count and are probably in general preferable.

TABLE 10

	First experiment		Second experiment	
	$\bar{x}_p$	$S(x_p^2)$	$\bar{x}_p$	$S(x_p^2)$
Santa Rita–1 (A)	6.40	6.00	6.04	4.96
Pike's Peak–4 (A)	5.92	5.84	6.08	7.84
Reedsport–2 (B)	5.48	6.24	5.64	5.76
Campbell R.–3 (B)	5.76	6.56	5.88	2.64

(c) *The Consistency of the Racial Difference.* We may finally inquire into the consistency of the difference in

external morphology between the races, as revealed by these measurements. We have two sets of data suitable for testing this point with reference to the sex combs.

In the first place it will be remembered that four strains, two of each race, were used in both of the experiments that were carried out. In each of these experiments the numbers of teeth in the proximal sex combs of 25 males were obtained (*cf.* Table 1). Now each of these counts was made on flies raised under the conditions described in Section 2, and in particular all were raised at 24.5° C. Hence both experiments should give substantially the same result for the racial difference in proximal sex comb teeth if the technique is suitable and the difference real.

The mean numbers of proximal sex comb teeth, based on 25 males, is given for each of these four strains in Table 10, as is the sum of squares of deviations of the individuals round the strain mean. There are two sets of values for each strain, one from each of different experiments. We may extract the desired information from the data by an analysis of variance. As there are four strains each comprising 25 individuals from each experiment, we have a total of 200 flies in all, and thus 199 degrees of freedom in the whole analysis. Of these 7 degrees of freedom will be concerned with the differences between the 8 means, and the remaining 192 will be concerned with the differences between individuals of the same strain on the same occasion. The 7 degrees of freedom between the means may be further subdivided into 1 for the difference between the two experiments, 1 for the difference between the races averaged over both experiments, 1 for the variation of the racial difference with the experiments, *i.e.*, the "interaction" of races and experiments, and 4 for the differences between strains of the same race and the interaction of these differences with experiments. These last four may be lumped, as we are not particularly interested in them. We use the arithmetical technique described particularly by Fisher (1936b) in analyzing the data and obtain the following analysis (Table 11):

TABLE 11

	Sums of squares	Degrees of freedom	Mean square
Experiments	0.02	1	0.02
Races	8.82	1	8.82
Race-Exp. Interaction	0.72	1	0.72
Strains and Strain-Exp. Interaction	4.60	4	1.15
Individuals	45.84	192	0.2388
Total	**60.00**	**199**	

It is clear that there is no real difference between the experiments nor is there any indication of an interaction between the racial difference and the experiments. In this way our belief in the reality of the racial difference in the proximal sex comb must be considerably strengthened.

The second test of the validity of the racial discrimination is in some way more stringent and of more interest. Samples of eight of the strains, four from each race, were raised at two different temperatures. The parents of the experimental flies were allowed to lay eggs at 24.5° C., as usual, and as before, were transferred to fresh bottles each day. When the eggs were laid, however, one set of bottles was allowed to develop at that same temperature of 24.5° C., and the results from these have, in fact, been incorporated in the analysis of Section 3 (*a*). Another set from the same parents was removed to a cold room with a temperature of approximately 17.5° C. These latter were subjected to temperature fluctuations of rather greater magnitude than is usual in an incubator, but even this variation is considerably smaller than the difference between the two temperatures used on the two sets of flies. Apart from the temperature difference the conditions, *e.g.*,

TABLE 12

		Cold			Warm		
		$\bar{x}_p$	$\bar{x}_d$	$\bar{X}$	$\bar{x}_p$	$\bar{x}_d$	$\bar{X}$
A.	Wawona–6	6.76	5.24	9.3480	6.72	5.08	9.2341
	Pike's Peak–4	6.60	5.40	9.2727	5.92	5.12	8.4539
	Tree Line–3	7.36	5.92	10.2898	6.56	5.56	9.3116
	Santa Rita–1	7.08	5.72	9.9108	6.40	5.16	8.9537
B.	Sequoia–5	5.96	5.56	8.7116	5.72	5.04	8.2143
	Reedsport–2	5.84	5.00	8.3145	5.48	4.20	7.5586
	Campbell R.–3	6.08	5.00	8.5545	5.76	4.80	8.1355
	Cowichan–6	5.36	5.08	7.8741	4.56	4.04	6.5594

number of flies per bottle, etc., were substantially the same.

The means of the proximal and distal comb teeth for all eight strains are given in Table 12. The cold series on the whole show more teeth per comb both proximally and distally. The variance of the discriminant suitable for combining the results of the two combs, as found in Section 3 (*a*), is analyzed in Table 13. There are 400 flies and hence 399 degrees of freedom in all. Of these, 384 are solely concerned with variations of individuals round the means of their strains. The difference between the warm and cold results takes up 1 degree of freedom, as do the racial difference and the interaction of the racial difference with the temperatures. The allocation of but 1 degree of freedom to the racial difference is not quite exact. It would be correct if the discriminant function used had been obtained from completely independent data. If the discriminant had been calculated from all the data, but no other, of these two experiments the racial difference would take 2 degrees of freedom. In actual practice half the data of this analysis, viz., the warm series, has formed $\frac{8}{23}$ of the results from which the discriminant was calculated. Hence neither 1 nor 2 degrees of freedom can strictly be allocated to the result. We shall not be far wrong, however, in taking the correct value as one, and in any case, as will be seen below, the results of the analysis are not seriously altered whether we take 1 or 2. There are 6 degrees of freedom for the variation of the strains round the racial means and also 6 for the interaction of this strain variation with temperature. This completely accounts for the 399 degrees of freedom of the analysis. We obtain the results shown in Table 13.

Again there is a large difference between the races whether 1 or 2 degrees of freedom are used, but it is now accompanied by a difference between the two experiments, as might be expected inasmuch as they were conducted at different temperatures. What is perhaps more impor-

TABLE 13

ANALYSIS OF VARIANCE OF THE X SCORE IN TEMPERATURE EXPERIMENTS

	Sums of squares	Degrees of freedom	Mean square
Experiments	53.6551	1	53.6551
Races	184.1832	1	184.1832
Race-Exp. Interaction	0.0202	1	0.0202
Strains	70.0283	6	11.6714
Strain-Exp. Interaction	12.3230	6	2.0538
Individuals	129.9914	384	0.3385
Total	**450.2012**	**399**	

tant, however, is that there is no evidence for interaction of the racial difference with temperature. In other words, the racial difference is not only real at both temperatures but it is the same at both temperatures. Neither is there any very large interaction between strains and temperature. Thus, in brief, we have found evidence of differences between the races of *Drosophila pseudoobscura* in the two sex combs, proximal and distal, and also in the wing and leg lengths of the male flies, though none was apparent between the females. Further, the racial difference in sex combs was successfully repeated on distinct occasions using the same cultural technique, and has also been shown to occur, and to be substantially the same, at two very different temperatures, one of which would be more favorable for the development of race A and the other for race B. Thus we can say quite definitely that there do exist differences in external morphology between the races, though it is true that the innate variation between individuals and strains obscures the racial difference unless a suitable statistical technique is employed in the analysis.

4. SUMMARY

The use of suitable statistical analyses permits the demonstration of racial differences in the numbers of the sex-comb teeth and in certain measurements of the legs and wings, when taken on male flies. No such differences were found between females of the two races.

The differences in numbers of sex-comb teeth were constant over a series of experiments even when performed at different temperatures.

The use of these criteria for assignation of strains to the two races would result in 10 per cent. misclassification.

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BUILDING STONES TO A CHEMISTRY OF EVOLUTION

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THE rule of inheritance is that like begets like. This holds for the largest as well as the smallest organisms. When an alga divides, there are two algae. There is twice as much water, twice as much minerals, twice as much protoplasm, enzymes, membranes, carbohydrates. For each molecule in the parent cell, there are two now. The same is ultimately true in the reproduction of higher plants and animals; we have a doubling (or trebling or multiple formation) of the parent organism, practically molecule for molecule. This is a most unusual event from the view-point of the chemist, because in a chemical reaction the original compounds never double themselves; quite to the contrary, they disappear and something entirely different appears in their place.

Evolution is a deviation from inheritance. As ordinarily interpreted, it means (at least ultimately) the development of more complex organisms from simpler ones. This can be brought about only by a change in the hereditary units. Evolution, then, means the acquisition of more hereditary units, and of new, different ones. The only hereditary units considered in this paper are the genes. The chemical laws which apply to them will very probably apply to other hereditary mechanisms. Evolution by loss of hereditary units is likely to be a rare case. Loss of genes does not necessarily require chemical reactions, and this possibility shall not be considered here.

Through irregular cell division, through fusion of wounded cells or by other means (*e.g.*, colchicinic acid), a cell may acquire more than its normal share of genes. A complete second set of genes or part of a second set may be added to a cell. This increase in the number of genes is caused primarily by a biological event whose frequency

of occurrence is determined by the frequency of irregular cell divisions, of wounding, etc. No chemical law can be applied to this phase of evolution.

The changes brought about by addition of parts of chromosomes or one or more entire sets of chromosomes are not very great, and the range of variation is limited. Great, decisive changes can be produced only by the creation of new genes. The making of a new gene is primarily a chemical event; the chemical reaction precedes the biological consequences. It shall be assumed here, for simplicity's sake, that a gene consists of one giant molecule (see P. Jordan, 1938). All chemical laws would apply also if the genes are multimolecular; the reaction would appear much more complex to us, but in nature, a multimolecular reaction proceeds just as easily as a unimolecular one.

Since the chemical composition of genes is entirely unknown, we can not say by what chemical mechanism the dividing cell manages to double all the genes. We only know that a gene once destroyed in a cell can never be replaced, and its offspring has to carry on forever without this gene (if the cell can live at all without it). This means that each gene can only be produced by itself, possibly with the assistance of other genes or protoplasm molecules. Without this gene, all other molecules in the cell can not reproduce it. The probability that a lost gene is reconstructed by the other molecules of the nucleus is zero, according to experimental genetics.

A gene must be an extremely complicated molecule. The creation of such a structure from simple food molecules is as improbable as spontaneous generation. Since untold millions of different genes have originated in all the plant and animal species since the beginning of life on earth, the formation of a new kind of gene must be possible. There is reason to believe that genes have a certain basic chemical structure in common. To the chemist, the appearance of a new gene indicates most likely a change of an already existing gene, the addition of some molecular group or

the loss of one. We might think of side chains in Ehrlich's sense. This chemical conception of the process which yields new genes agrees fortunately with the experiences of the biologist. Haldane (1932, p. 108) states:

> My own quite speculative theory of orthogenetic evolution . . . is that we are dealing here not only with the accumulation of genes having similar action, but with the very slow modification of single genes, each changing in turn into a series of multiple allelomorphs. The phrase "modification of the gene" is of course a rather misleading simplification. What I mean is that mutation was constantly modifying the gene, and that at any given time, natural selection acted so as to favor one particular grade of modification at the expense of others.

To such a chemical reaction of the gene, the law of mass action can be applied which states that the rate of reaction (here the frequency of a certain change) is proportional to the concentration of each reacting component. Only one gene in a cell will react at any time. The probability that two react simultaneously is very, very small. The other reacting component is unknown, though it must be a cell constituent. Since nothing is known about the other component, we must be satisfied with the statement that the rate of change of a gene is proportional to the concentration of that gene. Since there is only one gene of each kind per gamete, the rate can not be proportional to the nth power; it must be directly proportional to the gene concentration, except in polyploids.

Among the many factors influencing the rate of chemical reactions, temperature is quite important. An increase in temperature will accelerate the chemical process.

The rate may further be influenced by changes in the stratum where the reaction takes place. The change from light to dark is very important since light is capable of catalyzing many chemical reactions. The amount of available oxygen varies greatly at different depths of lakes and oceans. Rates of reaction are frequently affected by changes in concentration of electrolytes, especially of H ions, but of other ions as well, and even of non-electrolytes such as sugar, or protein.

We must expect, therefore, an increase in the creation of new genes from old: (1) by an increase in the concentra-

tion of genes on earth; (2) by an increase of temperature; (3) by a change of environment.

(1) The Rôle of Gene Concentration

The average cell has 2 equal sets of chromosomes, each with n genes. If an organism has x cells, it will have 2 nx genes and if there are y individuals of this species on earth, the "concentration" of all genes of this organism on earth is $2nxy$. All these chromosomes have a chance for variation, *i.e.*, for abnormal chemical reaction, but if such a change occurred in the somatic cells, it would be of no influence upon evolution in the case of animals, and very rarely only in plants. If it happened in gametes, it would be significant only in the case where this gamete developed into a new organism. Thus, the chance that a chemical change affects evolution is really proportional to the offspring produced over a certain period, *e.g.*, one year, or really to twice the offspring, since each gamete has equal chances.

According to Statistical Abstracts of the United States, 1934, there is in this country the following female population:

White	53,700,000 =	3,550	times	that	of	the	Chinese
Negro	6,035,000 =	399	"	"	"	"	"
Indian	162,047 =	10.7	"	"	"	"	"
Japanese	57,063 =	3.8	"	"	"	"	"
Chinese	15,152 =	1	"	"	"	"	"

Though there are slight differences in regard to number of children per mother, the above numbers represent fairly accurately the relative number of gametes which might undergo a chemical change. It does not represent the relative chances for mutation, for the rate of reaction in different races may be different. Certain races of Drosophila mutate much more easily than others.

Nevertheless, such numbers are instructive. The hens in U.S.A. laid 32,000,000,000 eggs in 1934, but only 673,000,000 chickens were raised. Consequently, the latter number is the factor for the chance of mutation.

According to Haldane (1932, p. 104), the number of wheat plants on earth is about 5×10^{14}, and this is the

factor for the evolutionary chance, not the 2×10^{16} seeds that were obtained from them. If we compare this number of plants with that of a rare orchid of which only perhaps 100,000 plants might exist on earth, the ratio is 5,000,000,000:1.

This is the significance of the concentration of genes on earth. If we assume that a gene in the rare orchid mutates once in 10 million plants, and in wheat much more slowly, perhaps once in 1,000 million plants, we would still have 500,000 mutations per year in wheat, and only one every century in the orchid. The concentration of genes, *i.e.*, the number of individuals of a species, is an important factor in evolution. When the differences in frequency of individuals are slight, this loses its meaning because of the many other factors affecting mutation, but when the differences are enormous, it must become significant. It should be possible to test this claim statistically. This can not be done with only a few species or families because different genes in different plants will have different rates of reaction. But if we consider all plants in the United States, this should be a number large enough to test the theory. Heller's "Catalogue of North American Plants North of Mexico" (1900) lists 16,673 species in 209 families. The families are arranged on the following pages according to the number of species.

Families with only 1 species: Ceratopteris, Mayaca, Croomia, Gyrotheca, Dioscorea, Casuarina, Leitneria, Apodanthes, Batis, Anredera, Tropaeolum, Hippocratea, Clusia, Canella, Koeberlinia, Carica, Datisca, Punica, Trapa, Symplocos, Sesamum, Phryma, Adoxa, Scaveola;

Families with 2 species: Trichomanes, Zamia, Canna, Marantha, Saururus, Anemiopsis, Peperomia, Schoepfia, Ximenia, Ceratophyllum, Podostemon, Crossosoma, Bursera, Melia, Swietenia, Pachysandra, Simondsia, Staphylea, Impatiens, Tamarix, Fouquieria, Amoreuxia, Piriqueta, Turnera, Pholisma, Ammobroma, Diospyros;

Families with 3 species: Osmunda, Pypha, Apteria, Burmannia, Butneria, Platanus, Empetrum, Corema, Ceratiola, Cyrilla, Cliftonia, Daphne, Dirca, Clethra;

Families with 4 species: Schizaea, Ornithopteris, Lygodium, Azolla, Salvinia, Halophila, Philotria, Phytolacca, Rivina, Petiveria, Menisperum, Cebatha, Hamamelis, Fothergilla, Liquidamber, Gordonia, Stuartia, Frankenia, Diapensia, Pyxidanthera, Shortia, Galax, Myrsine, Jacquinia, Icacora, Martynia;

Families with 5 species: Taxus, Tumion, Reseda, Oligomeris, Dipetalia, Elaeagnus, Lepargyrea, Menyanthes, Nephrophyllidium, Limnanthemum, Dipsacus, Knautia, Scabiosa;

Families with 6 species: Marsilia, Piluria, Triglochin, Scheuchzeria, Lilaea, Pontederia, Piaropus, Heteranthera, Buckleya, Nestronia, Comandra, Pyrularia, Simarouba, Suriana, Castela, Holacantha, Ailanthus, Picramnia;

Families with 7 species: Ephedra, Malpighia, Janusia, Aspicarpa, Thryallis, Byrsonimia, Tilia, Corchorus, Triumfetta, Elatine, Bergia, Terminalia, Conocarpus, Laguncularia, Bignonia, Chilopsis, Catalpa, Campsis, Stenolobium; Crescentia;

Families with 8 species: Najas, Eriocaulon, Dupatya, Lachnocaulon, Myrica, Comptonia, Sarracenia, Chrysamphora, Drosera, Dionaea, Parnassia, Aesculus, Plumbago, Statice, Limonium, Styrax, Mohrodendron.

Of the following groups, only the family names shall be mentioned:

Number of species per family	
9	Limnanthaceae
10	Sparganiaceae, Lauraceae
11	Moraceae, Melastomaceae
12	Callitrichaceae, Passifloraceae
13	Aizoaceae, Stemliaceae, Araliaceae, Monotropaceae
14	Lemnaceae
15	Zygophyllaceae
16	Selaginellaceae, Bromeliaceae, Sapindaceae
17	Urticaceae, Loranthaceae, Rhizophoraceae, Haloragidaceae, Sapotaceae
18	Juglandaceae, Loganiaceae
19	Xyridaceae, Ulmaceae, Nymphaeceae, Aceraceae, Pyrolaceae, Orobanchaceae
20	Lycopodiaceae, Magnoliaceae
23	Equisetaceae, Berberidaceae, Aquifoliaceae
24	Ophioglossaceae, Oxalidaceae
25	Fumariaceae
26	Rutaceae, Anacardiaceae, Cistaceae
27	Isoetaceae
28	Betulaceae, Aristolochiaceae, Hydrangeaceae, Celastraceae, Lythraceae, Lentibulariaceae
31 to 100	38 families

The theory claims that very common plants should show much variation, *i.e.*, many species, while very rare plants should show little variation, *i.e.*, few species. No strict parallelism can be expected. Many deviations will occur because each species has its own rate of mutation. But 16,000 species is such a large number that individual differences should not affect the grand averages. The difficulty of the proof lies in the impossibility of getting anything

The following are the families with more than 100 species:

Species	Family	Species	Family
105	Violaceae	258	Polemoniaceae
106	Pinaceae	266	Onagraceae
111	Gentianaceae	281	Cichoriaceae
116	Juncaceae	291	Boraginaceae
130	Asclepiadaceae	297	Caryophyllaceae
130	Chenopodiaceae	331	Umbelliferae
130	Orchidaceae	336	Labiatae
131	Rubiaceae	370	Polygonaceae
143	Solanaceae	373	Liliaceae
146	Salicaceae	373	Ranunculaceae
164	Ericaceae	529	Cruciferae
170	Polypodiaceae	558	Rosaceae
170	Saxifragaceae	627	Scrophulariaceae
189	Hydrophyllaceae	770	Cyperaceae
194	Cactaceae	1226	Papilionaceae
211	Malvaceae	1230	Gramineae
250	Euphorbiaceae	2631	Compositae

but a very rough and rather personal conception of the relative frequency of various species on a continent. Plants may be frequent in certain climatic regions or on certain soils, and entirely missing in the rest of the United States. It must further be remembered that with trees and perennials generally, only the number of new plants originating from seed can be counted. That eliminates most trees as "common plants" (Aceraceae, 19 species; Betulaceae, 28 species; Pinaceae, 106 species).

It will probably be admitted by most readers that practically all families with less than 10 species are rare, that the families with more than 1,000 species are the commonest plants, to be found in every locality on every soil.

Far more common than the commonest of all these plants are certain bacteria. *Bacterium coli* lives in the intestine of man and all mammals. It is excreted by man at the rate of about 25×10^{10} cells per capita per day, = nearly 10^{14} cells per year. Multiplied with the number of people on earth, this means more than 10^{23} individuals growing in man per year. To that must be added the growth in all mammals. Other intestinal bacteria (Streptococci, Lactobacilli, Bacteroides) will occur in numbers higher than 10^{20}. On the surface of all plants are regularly found *Bacterium herbicola* and *Pseudomonas fluorescens*. From the data given by Düggeli, the number of these bacteria on the surface of all plants in United States

must far exceed 10^{21} individuals. These numbers are 10 million to 1,000 million times that of all the wheat plants on earth. Among bacteria, we should expect the greatest frequency of mutations. That this is actually the experience of bacteriologists, to such an extent as to make taxonomy almost impossible, shall be shown in the last chapter.

The above-mentioned bacteria are not exceptions. There are millions of bacteria and thousands of protozoa in every gram of fertile soil; there are thousands of bacteria to every cc of surface water. Their rate of multiplication in nature is unknown. In this respect, the intestinal bacteria are probably exceptionally favored.

(2) The Rôle of Temperature

It is known to all physiologists that the rate of chemical reactions is increased by a rise of temperature, and that this law applies to reactions *in vivo* as well as *in vitro*. However, there is a striking exception in photochemical reactions which proceed at the same rate at all temperatures. This is quite important in our case, for it affords us the opportunity to test certain theories of the cause of evolution. In the preceding pages, the creation of new genes has been described as a chemical reaction. It has been frequently stated in recent years that radiation—be it ultra-violet, radioactive or cosmic in origin—may be the immediate cause of changes in the hereditary units of a species, and thereby the ultimate cause of evolution.

It seems possible to test this claim statistically. If evolution is caused by chemical changes of the common type which take place in the dark as well as in light, then evolution should go on more rapidly at the tropics than in moderate climates, and we should find the largest number of species of any one group near the equator. Exceptions would be mammals and birds whose temperature is independent of the climate. If, however, cosmic rays were the cause of evolution, temperature should have no great influence on species formation.

Of great interest in this respect is an investigation by Tischler (1934) into the frequency of polyploids in differ-

ent climates. Although the chromosomal composition of Sicilian plants is known for only one third of all species, and for Iceland and Faroe Islands for only about one half of the species, the following table is quite instructive.

	Square kilometers	Latitude	Number of species			Percentage of polyploids		
			Dicots	Monocots	Total Angiosperms	Dicots	Monocots	Total Angiosperms
Iceland	103,000	66–70°	239	120	359	47.4	71.9	54.5
Faroe Isands	1,400	63°	172	95	267	42.7	63.5	49.4
Schleswig-Holstein	23,000	54–55°	777	293	1,070	39.0	60.0	44.1
Sicily	25,000	37–38°	1,800	545	2,345	26.0	48.8	31.3

The left half of the table shows a decided increase in the number of species with a decrease in latitude, *i.e.*, with an increase in temperature. This suggests that the creation of new species is favored by an increase in temperature. The right half of the table shows that polyploidy (which has been explained above as not being caused by chemical reactions) is not favored by an increase in temperature, but actually increases distinctly in the colder climates. This was also emphasized by the fact that those plants of Schleswig-Holstein which are found also in northern countries consist to 60 per cent. of polyploids (127 species) while those which are found in southern countries contain only 27 per cent. polyploids (70 species). Whether the cause of polyploidy be cosmic radiation or an adaptation mechanism for growth at lower temperatures, the cause is evidently different from the main species-forming force of evolution, which is chemical in nature and increases in activity with higher temperatures.

It might be doubted that the regions selected are large enough to use them for such statistical surveys. Since the angiosperms represent by far the largest part of the flora of any country, they may well be considered representative. A second example shall be chosen from zoology, namely the reptiles.

This group has been chosen because it is compiled quite comprehensively in Ditmars's book, "Reptiles of the World" (London, 1910). Though quite a large number of species have not been given in sufficient detail, there was no doubt in the large majority of cases whether a species lived in a temperate or a hot climate. In doubtful cases, the decision was made in favor of temperate climate. South Africa and southern South America were considered temperate, also California and New Mexico, while Mexico and southern Europe were considered hot. The result of the compilation was as follows:

	Number of species		
	In moderate climates	In hot climates	Undecided
Chelonia (turtles)	50	135	30
Crocodilia (alligators)	0	23	0
Lacertilia (lizards)	120	1,408	89
Ophidia (snakes)	165	1,219	226
Total reptiles	335	2,785	345
Percentage distribution	10 per cent.	80 per cent.	10 per cent.

Even if all the undecided species were counted as belonging to the moderate climates, they would still represent less than one fourth of those in hot climates. The agreement with Tischler's statistical results with plants is rather convincing.

I have been told by zoologists that a similar, probably even greater majority of species in hot climates exists in the fishes and among insects, but I have found no material that could be treated without a very great amount of work. The difference should not be so great among the warm-blooded animals, the mammals and birds, because the genes are kept at uniform body temperature.

An analysis has been attempted by classifying the mammals listed in Trouessart's "Catalogus Mammalium," 1898–9. The result is at first glance not much different from that of the reptiles for it shows a distinct majority of tropical species. But with reptiles, the species of moderate climates averaged 10 per cent., and with mammals 26 per cent. of the total. The outspoken dominance of

DISTRIBUTION OF ALL MAMMALS BETWEEN TROPICAL AND TEMPERATE CLIMATES

	Number of species					Percentage of living species		
	Extinct	Tropical	Moderate	Undecided	Total living species	Tropical	Moderate	Undecided
Bimana	..	..	..	1	1	..	..	100
Primates	28	207	4	17	228	91	2	7
Prosimia	85	49	1	1	51	96	2	2
Chiroptera	36	396	42	83	521	76	8	16
Insectivora	72	160	84	30	274	58	31	11
Carnivora	516	143	111	68	322	44	35	21
Pinnipedia	23	2	21	7	30	7	70	23
Rodentia	435	759	525	184	1,468	52	36	12
Tillidontia	38	0	0	0	0	0	0	0
Ungulata	1,428	201	79	70	350	57	23	20
Sirenia	31	6	1	0	7	86	14	0
Cetacea	290	26	51	35	112	23	45	32
Edentata	334	42	2	6	50	84	4	12
Marsupialia	248	84	27	73	184	45	15	40
Allotheria	49	0	0	0	0	0	0	0
Monotremata	10	1	0	2	3	33	0	67
Total mammals	3,623	2,076	948	577	3,601	58	26	16

tropical species in mammals is limited to the monkeys and the bats, about one fourth of all species, while the snakes, lizards and crocodiles represent 94 per cent. of all reptiles. The table comparing only those species with outspoken climatic preferences, shows this difference very distinctly. The grand average ratio of species in tropical and moderate climates is with mammals 69:31, *i.e.*, 2:1 and with reptiles 9:1.

A preponderance of species in tropical climates must be expected even with mammals for the simple reason that with the abundant food, the number of individuals is considerably larger. The effect of the number of individuals on the frequency of variation has just been discussed. While the above evidence in regard to mammals can not be considered absolute proof, the ratio of species in hot and cool climates, which is so very different with cold-blooded and warm-blooded animals, suggests very strongly a strong influence of temperature upon the rate of evolution.

(3) THE RÔLE OF ENVIRONMENT

It is generally assumed that the slow geological changes causing a gradual change of climate, together with the

RATIO OF SPECIES IN TROPICAL AND TEMPERATE CLIMATES, AFTER OMISSION OF UNDECIDED CASES

Mammalia				Reptilia			
Species considered		Tropical	Temperate	Species considered		Tropical	Temperate
1	Monotremata ...	100	0	23	Crocodilia	100	0
50	Prosimia	98	2				
211	Primates	98	2				
44	Edentata	95	5				
438	Chiroptera	91	9	1,528	Lacertilia	92	8
7	Sirenia	86	14	1,384	Ophidia	88	12
111	Marsupialia	76	24	185	Chelonia	73	27
280	Ungulata	72	28				
244	Insectivora	66	34				
1,284	Rodentia	59	41				
254	Carnivora	56	44				
77	Cetacea	34	66				
30	Pinnipedia	9	91				
Average		69	31	Average		90	10

glacial periods, have been important factors in evolution. When a moist climate gradually becomes arid, the cell sap in plants may become more concentrated and thus cause a reaction which was impossible before. The decrease of fog and clouds may permit very short ultra-violet light to strike the seeds of plants or eggs of insects, and a reaction may thus take place hitherto unknown in that group of organisms. Such climatic changes may be sudden, as the drying of a swamp or salt marsh, but usually require many thousands or even millions of years.

Similar and even greater changes occur to some kinds of microscopic organisms far more frequently. Each gram of soil differs from the neighboring gram in its oxygen supply, in the amount of mineral and organic matter, in moisture content and exposure to sunshine. A large plant extends its roots over many cubic feet of soil and obtains the average effect, but to a microscopic plant like an alga, one gram is a universe. This soil climate of the alga will change with every rain, with every dry day, with a leaf falling on the ground. The algae, bacteria and protozoa of the soil will undergo continuous drastic changes of life conditions, and the chances for an abnormal reaction of any one of the genes is greatly increased.

An even greater change occurs daily at a stupendous scale to the bacteria commonly inhabiting the surface of plants, *Bacterium herbicola, Pseudomonas fluorescens* and in smaller quantities some of the *Lactobacilli* and *Streptococci* and other genera. They are swallowed by herbivorous animals at the rate of a hundred thousand individuals per gram of plant substance. This means a change from the dry, brightly lighted environment with abundant oxygen, but scant food on the outside of the leaves, to the dark, completely anaerobic intestine with an abundance of soluble food. There the bacteria remain for 1 to 7 days, depending upon the species of animal, and most of the bacteria die. The few survivors are thrown out, onto the soil or into water, together with an enormous number of intestinal bacteria which had been completely adapted to the uniform temperature and the rich food in the intestine, and which are bound to die in the new environment unless they can adapt themselves. This same change applies also to the infusoria swallowed by animals with the drinking water, and to the normal or pathological protozoic fauna of the animal intestine. This change of environment happens to untold billions of bacteria daily. It is only natural to suppose that entirely different food constituents passing through the cell membrane to the growth centers of the bacterial cell might occasionally, perhaps once in a trillion individuals, cause a variation in one gene, and thus produce a new variety.

Taxonomy of bacteria seems almost impossible because of the existence of nearly any imaginable intermediate form between any two established "species" (Rahn, 1920, 1928). We must either assume that these intermediates found "climates" or "universes" where they could multiply ever since they were first created or that they arise now and then by mutation from the "standard species." The latter assumption is far more probable, and can be supported experimentally.

Bacteria are so small and so simple in their forms that distinction of species within the genus is based largely on

biochemical properties, such as the ability to digest gelatin, or to ferment starch, sucrose, lactose, galactose, etc. These biochemical properties have been frequently found to vary. One of the first carefully studied cases is that of *Bact. coli mutabile* (Neisser and Massini). This species does not ferment lactose, and therefore produces white colonies on Endo agar. When these colonies are kept for a number of days, secondary colonies become visible in some of the old white colonies, and these new tiny colonies are red, *i.e.,* they can ferment lactose. The progeny from the red colonies "breeds true," it keeps the ability to ferment lactose even after many transfers on lactose-free media. The offspring of the white colonies continues to produce white colonies, with an occasional red secondary colony. This sounds like a simple case of occasional mutation of a single cell, and the frequency is about 10^{-9}.

Quite similar is the observation of Twort with typhoid bacteria. While *Bact. typhosum* in the ordinary fermentation test will not attack dulcitol, it can be "trained" to ferment this sugar merely by cultivating it in dulcitol broth. Some strains will start to ferment this sugar after the third transfer, others not until the tenth transfer. The cultures then retain this ability for many transfers. The plate counts of the cultures suggest that a single cell, or a very few, acquire suddenly this ability and thus having more food available, outgrow the old strain.

While all strains of *Bact. typhosum* learned to ferment dulcitol, only one of all the strains tested by Twort and later by Penfold learned to ferment lactose. This is a very rare mutation, happening only perhaps once in 10^{12} individuals.

It is not proved, however, that these mutations are really caused by the continuous chemical stimulation of the sugar concerned. It may be that the same mutation would have happened anyway, even in the absence of the special sugar; but since the sugar was present, the new sport was better nourished and outgrew the unaltered cells. Lewis's evidence points in this direction. He grew a different strain

of *Bact. coli mutabile* on a sugarfree agar, and found that some of the colonies, about 1 of 500,000, were capable of fermenting lactose. The proof is not absolute because no test is possible without bringing bacteria in contact with the sugar, and then, adaptation may occur. Sherman's experiments with the variation of Streptococci also seem to indicate a rather easy and spontaneous acquisition and loss of ability to ferment certain sugars.

This same logic has been employed to mutations produced by climatic changes through geological causes. Mutations may happen regularly, and independently of climate or environment, and only when the environment changes, may the mutant outgrow the original form.

For the study of chemical (and physical) factors in evolution, bacteria have decided advantages, but also great disadvantages, and it will depend upon the purpose of the experiment whether or not bacteria can be used.

While the geneticist works with hundreds and thousands, rarely with millions of individuals, the bacteriologist finds many million cells in each cc of his culture medium, and one liter of culture frequently contains 10^{12} individuals. Such a population exceeding the human population of the world can be produced in 24 hours.

There are disadvantages in such large numbers. Only very small samples of such population can really be studied. The observation of lethal mutations would be absolutely impossible. A mutant which grows more slowly than the average is soon completely crowded out and will never be observed. A mutant which grows more rapidly than the rest will soon crowd out the original species.

A very extensive literature relates of such changes, morphological as well as physiological, and often the changes are so that the mutant represents not only a different species, but a different genus, and occasionally, even a different family, *e.g.*, the permanently irreversible asporogenous variety of *Bacillus anthracis* would, if found outside of an animal, have to be classified under *Bacteria-*

ceae, not *Bacillaceae.* This mutation can be brought about by continued cultivation on glycerol agar. Other striking changes are produced by cultivation on media with dyes, such as methyl green or malachite green, or with disinfectants like phenol or trichloracetic acid. *Bacterium coli* has thus been changed to a bacterium which can no more form gas from sugar, and it would never be recognized as a variant of *B. coli* if isolated from water or milk.

This list of mutations could be extended over many pages. Most of these changes enforced by severe chemical treatment represent losses of properties, *i.e.,* destruction of genes rather than syntheses. They form a great contrast against the acquisition of new fermenting properties. By a combined loss of some properties and acquisition of others, the bewildering variety of all possible combinations of properties which makes taxonomy of bacteria next to impossible, is easily explainable.

This discussion does not include the much discussed changes from "smooth" to "rough" and "mucoid" and "G" forms of bacteria which are of great practical importance, but probably do not belong in this discussion. The author agrees with Hadley (1937) that we are dealing here with different manifestations of morphology and physiology whose range is not known in its entirety, but does not affect hereditary units.

Summary

The object of this paper was to point out that evolution depends to a large extent on the formation of new kinds of hereditary units, *e.g.,* genes. Such formation can be caused only by a chemical reaction, and therefore must follow chemical laws. While the actual reagents involved are entirely unknown, some general laws can be applied successfully, and seem to justify the following statements:

The frequency of the creation of a new hereditary unit in any given species is proportional to the number of individuals born per year. This is borne out by the evidence that of the plants of North America, the rare families have

few species, and the common families have many species.

The frequency of the creation of a new hereditary unit in any given species is greater in warmer climates because chemical reactions proceed more rapidly at higher temperatures. This is borne out by the evidence that among reptiles, there are about eight times as many species in tropical climates as in moderate climates, while with mammals whose temperature is constant, the ratio is only 2:1. The number of species of plants also increases with the temperature of the country.

This evidence also indicates that the formation of new hereditary units is not caused by cosmic rays or any other kind of radiation, because the rate of reactions caused by radiation is independent of the temperature, and statistical evidence shows a dependence.

Polyploidy is not caused by chemical reactions, and it shows no relation to the number of species existing, nor does it increase with increasing temperature.

A change of environment is likely to affect cell chemistry, and to induce new reactions which might lead to the formation of new hereditary units. The frequency of such reactions should be proportional to the frequency of environmental changes. This is largely a question of size. Some microscopic organisms may change their environment daily on an enormous scale, *e.g.,* intestinal and soil bacteria. Variation in bacteria is so common that in several groups, species definitions are absolutely arbitrary because all shades of intermediate forms between any two "established" species have been described.

While the attempt has been made to collect for each point some convincing material, the author realizes that much more material must be treated statistically to prove the suggestions made in this paper. The author invites biologists to do this, since he expects to limit his own studies to bacteria.

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SEX RATIOS AND TWIN PRODUCING KINDREDS

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INTRODUCTION

IT seems to have been well established through the work of Davenport (1920a, b, c, 1927), Curtius (1927), Dahlberg (1926, 1930), Greulich (1934), Hamlett (1935), Fisher (1928), von Verschuer (1932), Danforth (1916) and many others that the tendency to produce twins is of a hereditary nature, although there seems to be some difference of opinion concerning the nature and extent of this hereditary influence. On the one hand, Weinberg (1901, 1909, 1927), Bonnevie (1919, 1926), Eckert (1928) and Wehefritz (1925) seem to have evidence that indicates that only the dizygotic type is hereditary and that the monozygotic type occurs spontaneously in all cases. On the other hand, Davenport, Curtius, Dahlberg, Fisher, Greulich and others have presented evidence that clearly demonstrates a hereditary basis for both types of twinning. Curtius (1927) and Dahlberg (1930) conclude from their studies that both monozygotic and dizygotic twins occur in the same families and often in the same fraternities, while Bonnevie (1926) and Greulich (1934) conclude from their studies that, in the words of Greulich, "dizygotic and monozygotic twinning are not different expressions of the same twinning tendency, but on the contrary, seem to be phenomena quite distinct from each other."

Some further information that seems to bear on this general subject has developed from an attempt to separate kindreds[1] into groups on the basis of the type of twin represented in each. To this end, it was planned to make the

[1] A *kindred* is defined as a group of individuals each of whom is related in some way, either by blood relationship or by marriage, to every other member of the group and, in so far as is or can be known at any given time from the available records, to no other person.

separation in accordance with the fact that all kindreds which possessed one or more pairs of unlike sex twins would *certainly* possess factors for the determination of fraternal twinning. If the factors for the production of the two types of twins were entirely independent of each other, one would expect, in view of the low general incidence of .3912 per cent. for monozygotic twinning and of .7587 per cent. for dizygotic twinning, according to the figures of Greulich (1934), that there would occur only a very occasional instance when the factors for the production of identical and those for the production of fraternal twinning would occur coincidently in any one kindred. It seemed therefore that one might expect to be able to separate a given group of kindreds into two groups, one of which would represent practically all the occurrences of the factors for the production of fraternal twins, while the other would represent practically all the occurrences of the factors for the production of identical twins.

Material and Analysis

The studies were conducted upon all the kindreds containing 25 or more individuals which were filed under Trait 053 in the "A" File of the Eugenics Record Office. It was believed that kindreds of such size would provide kinship data of such quantity as would allow of further study, whereas smaller kindreds would have so few of such data as to make analysis very difficult and complex, if not impossible. Aside from this single consideration, the data of the archives were left unselected. It seemed that such an unselected group could be considered to represent a random collection of identical and fraternal twins in their typical and various relationships.

There were, in all, 85 kindreds of this size, and these contained a total of 919 twins. Of these twins, 26 pair were of unknown sex, 339 pair were of unlike sex, while 554 were of like sex. According to the difference method of Weinberg (1901, 1909) for calculating the number of identical and fraternal twins, and ignoring the 26 twins

of unknown sex, one may determine that there are $893 - 678 = 215$ identical twin pairs in the population. This gives 24.1 per cent. identical to 75.9 per cent. fraternal twins. Dahlberg (1926, pp. 12–21) gives a detailed description of the history of the development and evaluation of the difference method, both before and after Weinberg's description (1901).

It is, of course, impossible to determine the effect that the twins of unknown sex would have upon the calculated frequency of identical twinning if the sexes could be known, but it is certain that though the proportion of identical twinning is slightly lower than the value of 26 per cent. cited by Newman (1937) based on data from a twin population of 717,907 twins provided by J. B. Nichols, and similar values provided by numerous other workers, it is still of sufficient magnitude to indicate the presence of a rather definite proportion of identical twins in this population. Eight triplets also occurred in the group, 2 of like sex, 5 of unlike sex, and 1 of unknown sex.

Further analysis of this group has shown that, among the 85 kindreds used in the study, some of the twins were specifically described with sufficient detail to enable one to determine whether twins (of like sex) were identical or fraternal in 14 of these kindreds. Of such descriptions, however, 22 pair were represented, of which 14 were identical and 8 fraternal. On the basis of the 75.9 per cent. fraternal twins and 24.1 per cent. identical twins found as described for the population as a whole, one would expect approximately 25 identical to 37 fraternal like sex twins, and, although the greater interest that exists in identical twins may be expected to result in a more frequent description of the occurrences of such twins, one may nevertheless regard the ratio of 14 identical to 8 fraternal twins as being a good indication that there is no unusual absence of identical twins in this population as a whole. In these characteristics, this group compares favorably with the groups that formed the bases for the earlier studies.

Since, however, it seemed that if the processes which underlay the production of these two types of twins were such as to render the causes of their production entirely independent, then one might expect that the coincident chance occurrence of both types of twinning, in view of the low general incidence of twinning in the general population of 1.15 per cent. according to Greulich (1934) as rendered in effect even lower by the high familial incidence of the trait as demonstrated by Davenport (1920a, b, c, 1927), Greulich (1934) and several others, would be very rare indeed. This postulated rarity of the expected occurrence of such coincidences led to a tentative working conclusion that it should be possible to separate the identical from the fraternal twins by making a selection upon the basis of the presence or absence of twins of unlike sex within a kindred. All kindreds which contain one or more twins of unlike sex would *positively* possess the factors for the production of fraternal twins, while, in the light of the foregoing, only in very rare instances would they contain factors for the production of identical twins. On the other hand, though the kindreds which contain only like sex twins might contain factors for the production of fraternal twins according to the laws of chance distribution, it would be expected that, by process of elimination, by far the larger proportion of such kindreds would contain only the factors for the production of identical twinning. The cases of coincident occurrence of factors for the production of both types of twins would be just as rare as in the group of kindreds where unlike sex twins occur.

On this basis, one would expect that the resultant distribution of twins would be such that practically all fraternal twins would occur in kindreds which contained one or more pairs of unlike sex twins, and that practically all the identical twins would occur in kindreds which contained only like sex twins. In view of the earlier determination, one would expect that approximately 24 per cent. of the total number of twins of the group, or 215 twins, would occur in kindreds which contain only like

sex twins, while approximately 76 per cent. of the total, or 678 twins, would occur in kindreds that contain one or more unlike sex twins.

The separation was made on this basis, and, quite contrary to these expectations, it was found that but 6.05 per cent., or 54 pair of twins, occurred in kindreds which contained only like sex twins, while 93.95 per cent., or 839 pair, occurred in kindreds that contained one or more pairs of unlike sex twins.

It might be reasoned that this condition was due to a failure in the reporting of all the cases of births of unlike sex twins caused by a lessened degree of interest in such twins, but this explanation could not possibly account for the *small* number of kindreds where only twins of like sex occur, while more accurate recording might even reduce the number of such kindreds. It is obvious, too, that it is not the relative numbers, but rather the relative *distribution* of the unlike sex twins that produces these conditions, and one would, therefore, not expect it to be due to a selective sexual selection, either in the form of a differential intrauterine mortality or in the form of sex reversals and intersexuality produced by a fusion of the circulatory systems in unlike sex twins. It is, indeed, difficult to conceive of a process whereby unlike sex twins could develop originally from identical or fraternal like sex twins, and the reverse process, though, of course, feasible, could not possibly effect the redistribution of the unlike sex twins required by the foregoing expectations.

It is, however, possible, though it seemed very improbable, that the condition could be produced by the simultaneous chance occurrence of the two types of twinning in a few of the large kindreds produced by a fusion by marriage of an original identical twin producing kindred with an original fraternal twin producing kindred. This might, in one such fusion, involve kindreds of sizes sufficient to account for the entire disturbance in the expected distribution among kindreds of like and unlike sex twins. In such a case, however, it would be expected that the

entire disturbance would be confined to but one, or at most, to but two very large kindreds, and that it should be possible readily to discern the two subgroups within the kindreds. As is shown in the following analysis, it has not been possible to do this.

Of the kindreds that formed the material for this study, the five largest contained 120, 93, 49, 35 and 33 twins, respectively. Five contained between 20 and 30 twins, while nine contained between 10 and 20, 24 contained between 6 and 9, 14 contained 5, and 28 contained between 1 and 4. The five largest of these kindreds were examined in order to determine if the distribution of the twins could be produced in the way already outlined.

These kindreds contained a total of 330 twins, or 37 per cent. of all the twins considered in the study, and of these, 125 were of unlike sex, 102 were male twins, and 103 were female twins. This would give an excess of like sex twins of $103 + 102 - 125 = 80$, or very close to 37.2 per cent. of the 215 excess like sex twins in the total population. Certainly, there is no sufficient massing of the like sex twins in any of these larger kindreds to account for the distribution of the twin types found.

Nor is any massing apparent in any of the other kindreds used in this study, as may be seen from a study of Table I, where the number of male twins, the number of female twins and the number of unlike sex twins are presented for each of the kindreds considered. This table is presented in five sections. In the first are grouped the 43 kindreds which have more like than unlike sex twins, in the second, the 14 kindreds having no unlike sex twins, in the third, the 14 kindreds having more unlike than like sex twins, in the fourth, the 10 kindreds having equal numbers of like and unlike sex twins, and in the fifth, the 4 kindreds having only unlike sex twins. Comparison of these groups shows that a total of 682 twins, or approximately 76.5 per cent. of all the twins, occurred in kindreds where there are more like than unlike sex twins. It is true, to be sure, that a large portion of these twins occur

TABLE I

RELATIVE OCCURRENCE OF THE TWIN TYPES IN THE KINDREDS HAVING MORE THAN TWENTY-FIVE INDIVIDUALS, ARRANGED IN FIVE GROUPS

Group I. More like than unlike sex twins

Kindred reference	Number of twins			
	Like sex		Unlike sex	Total
	Male	Female		
39	4	6	5	15
40	2	3	2	7
42– 43	2	1	2	5
73	5	2	5	12
80	2	3	2	7
81	3	2	1	6
82	2	4	2	8
83	1	2	2	5
84– 85	1	1	1	3
86	2	2	1	5
97– 98	1	5	1	7
101	1	3	1	5
102	1	2	1	4
106–110	3		2	5
120–121	4	2	1	7
132–133	5	4	6	15
137–138	2		1	3
140	32	38	50	120
165–173	4	6	5	15
184	1	3	1	5
189–191	5	3	1	9
197	1	5	1	7
210	2	3	1	6
230	10	13	10	33
373–374	3	1	1	5
407–414	7	4	5	16
415–424	4	4	6	14
425–432	10	5	12	27
433–439	7	9	12	28
460–464	3	1	2	6
465–468	3	1	2	6
469–475	7	5	10	22
476–483	14	7	14	35
484–487	7	4	10	21
493–506	18	12	19	49
507–511	3	7	5	15
512–536	28	33	32	93
538	1	1	1	3
539–542	2	3	4	9
700–701	1	3	1	5
702	1	4	1	6
818–820		2	1	3
835–837		3	2	5
Total 43	215	222	245	682

in the larger kindreds of the whole group, but even if one consider only the kindreds that have less than 10 twins, he finds that there are, in this group, 152 twins, or 48 per cent. of all the twins that occur in such kindreds. By confining consideration to such kindreds, sixteen kindreds representing 530 twins are excluded from the group having more like than unlike sex twins, while a total of but 3 kindreds representing but 48 twins are excluded from the remaining four groups.

TABLE I—(*Continued*)

Kindred reference	Number of twins: Like sex, Male	Like sex, Female	Unlike sex	Total
Group II. Only like sex twins				
75	6	4		10
99		2		2
105	2	4		6
250–274		1		1
275–278	5	2		7
356–357	3	1		4
381–382		2		2
488–492		3		3
638		3		3
641		2		2
643	1	1		2
646	1	2		3
659	4			4
797–798	4	1		5
Total **14**	**26**	**28**		**54**
Group III. More unlike than like sex twins				
1		1	3	4
76– 77		1	2	3
78– 79	3		4	7
88		1	2	3
95– 96	1		4	5
139	2	1	6	9
141–142	1	2	6	9
245		1	3	4
371–372	1	1	3	5
440–451	9	3	14	26
636–637	2		3	5
725–727		2	3	5
791–793	1	1	5	7
770–771	1		3	4
Total **14**	**21**	**14**	**61**	**96**
Group IV. Equal number of like and unlike sex twins				
89– 94	1		1	2
131	2	2	4	8
279–283	1	2	3	6
452–459	2	4	6	12
537		1	1	2
626–629	1	1	2	4
650–651	1	2	3	6
653	2	1	3	6
740–741	3		3	6
759–760	1	1	2	4
Total **10**	**14**	**14**	**28**	**56**
Group V. Only unlike sex twins				
198			1	1
639			2	2
658			1	1
784–785			1	1
Total **4**			**5**	**5**

The distribution of the twin types in the retained kindreds is presented in Table II, where the total number of kindreds, the total number of twins, the number of male, female and unlike sex twins corresponding to each twin composition of the kindreds is presented. In a group of kindreds of sizes varying symmetrically about an average

TABLE II

OCCURRENCE OF THE TWIN TYPES AMONG THE FIVE KINDRED SUBGROUPS IN THE GENERAL GROUP OF KINDREDS WHICH HAVE LESS THAN TEN PAIRS OF TWINS

Twin composition of kindred subgroup	Number of kindreds	Number of twins			Total
		Like sex		Unlike sex	
		Male	Female		
More like than unlike sex twins	27	50	63	39	**152**
Only like sex twins	13	20	24	..	**44**
More unlike than like sex twins	13	12	11	47	**70**
Equal number like and unlike sex	9	12	10	22	**44**
Only unlike sex twins	4	..	..	5	**5**
Total	**66**	**94**	**108**	**113**	**315**

of approximately 4.75 twins per kindred, as may be seen in Fig. 1, one would expect, on the basis of chance distribution of fraternal twin types, an essentially equal number of twins to occur in kindreds where more like than

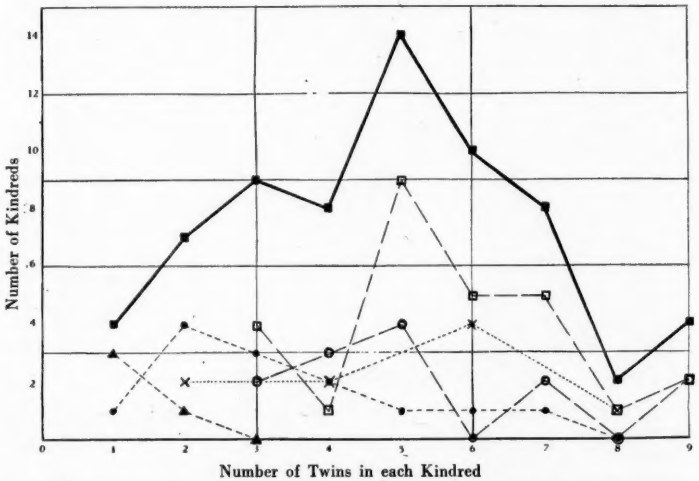

FIG. 1. Graph showing frequency distribution of kindreds on the basis of number of twins that appear in each. The distribution of all kindreds is shown by solid squares, while the five subdivisions of this total group are shown by circles and squares for those having more unlike than like, and those having more like than unlike sex twins respectively. Solid triangles and circles represent those having only unlike, and those having only like sex twins respectively, while crosses represent those having equal numbers of like and unlike sex twins.

unlike sex twins occur and in kindreds where more unlike than like sex twins occur, and an essentially equal number of twins to occur in kindreds where only twins of unlike sex occur and in kindreds where only twins of like sex occur. The excess of twins in the latter group, or $44-5=39$ twins, should represent approximately the number of identical twins in the total population. These 39 twins represent, however, but 12.5 per cent. of the total number of twins, although the excess of like sex, and so, presumably, of identical twins is $315-226=89$, or 28.4 per cent. On the other hand, in the other two groups of kindreds, there is a total of 86 twins of unlike sex. These call for an equal number of like sex twins, which leaves $222-2(86)=50$ excess like sex twins in kindreds where unlike sex twins occur.

It is therefore obvious that the unexpected distribution of the twin producing kindreds on the basis of presence or absence of unlike sex twins is *not* produced by an occasional simultaneous chance occurrence of the two types of twinning in the same kindred. Instead, it is shown that the unexpected distribution occurs rather widely and in a large majority of the kindreds, irrespective of their size.

On the other hand, however, the condition is not merely that of a natural random scatter of identical twins, as can be readily shown from already available statistical data. Thus, taking the figure of 1.15 per cent. of the U. S. Census Report for the incidence of the occurrence of twins in the general population, and using the figure of 24 per cent. to represent the portion of these that are believed to be of the identical or uniovular type, then, if one assume that the identical twins occur entirely independently of the fraternal twins, it would follow that the random distribution of such twins would have an incidence of occurrence of approximately $.24(1.15)=.276$ per cent., or about 276 in 100,000 births. One would expect, on this basis, about 2.465 identical twins to occur in the group of 893 twin births, and one obtains essentially the same results if he assumes that identical twinning is hereditary. In

such a case, the identical twins would occur in groups of two or more, but the incidence for the occurrence of the groups would be correspondingly less. Certainly, chance coincidence of small groups of identical and fraternal twins in the same kindreds could not occur frequently enough to account for the distributions that have been found.

It seems from this, then, that one is forced to conclude that the factors for the determination of identical twinning and those for the determination of fraternal twinning are *associated* in some way. Final conclusive proof of the occurrence of this association is provided by a further study of the 22 pair of like sex twins that occur in the 85 kindreds of this study for which *specific* descriptions regarding their type are provided. Of these, but four pair of identical twins occur in kindreds in which no fraternal twins or twins of unlike sex occur, while in one kindred in which only like sex twins appear, two pair of fraternal twins occur. On the other hand, specifically described identical twins occur in seven kindreds representing 10 pair of twins in which twins of unlike sex occur. In two of these kindreds representing three pair of identical twins, three pair of like sex fraternal twins are also specifically described. Three like sex fraternal twins with no identical twins are described in three kindreds in which unlike sex twins also occur. These conditions are shown in Table III.

And final confirmatory evidence for the association between the factors for the production of identical twins and those for the production of fraternal twins comes from a study of the smaller kindreds filed in the 053 series of the "A" File of the Eugenics Record Office Archives. In this group, there occurred 108 kindreds which contained specifically described identical or like sex fraternal twins. Forty-nine of these contained 2 or more twins, and 24 of these contained one or more identical twins. Of these 24 kindreds, 15 positive cases representing 62 per cent. of these kindreds presented an occurrence of identical twins

TABLE III

OCCURRENCE OF THE SPECIFICALLY DESCRIBED IDENTICAL AND LIKE SEX FRATERNAL TWINS AMONG THE KINDREDS HAVING MORE THAN TWENTY-FIVE INDIVIDUALS, IN RELATION TO EACH OTHER AND TO THE UNLIKE SEX TWINS OF THE GROUP

Kindred reference	Specifically described twins				Undescribed twins			Total
	Identical		Fraternal		Like sex		Unlike sex	
	Male	Female	Male	Female	Male	Female		
73.....	2				3	2	5	12
131.....	1				1	2	4	8
106–110.....	1		2				2	5
120–121.....	2		1		1	2	1	7
373–374.....	2				1	1	1	5
818–820.....		1				1	1	3
835.....		1				2	2	5
275–278.....	1	1			4	1		7
381–382.....		1				1		2
797–798.....		1			4			5
356–357.....			2		1	1		4
245.....				1			3	4
740–741.....			1		2		3	6
759–760.....			1			1	2	4
Total 14	9	5	7	1	17	14	24	77

TABLE IV

OCCURRENCE OF SPECIFICALLY DESCRIBED IDENTICAL TWINS IN KINDREDS WHICH, IN THE FIRST SECTION, ALSO HAVE ONE OR MORE PAIRS OF LIKE SEX FRATERNAL OR UNLIKE SEX TWINS, AND, IN THE OTHER SECTION, HAVE ONLY IDENTICAL OR UNDESCRIBED LIKE SEX TWINS

Kindred reference	Specifically described twins				Undescribed twins			Total
Section I	Identical		Fraternal		Like sex		Unlike sex	
	Male	Female	Male	Female	Male	Female		
19.....		1		1			1	3
112–115.....		1		1			1	3
216–217.....		1					1	2
375–376.....		1				1	1	3
379.....	1						1	2
709–710.....	1					1	2	4
711–712.....		1					1	2
739.....		1			2		1	4
754–755.....	1				1		1	3
765.....		1			1		2	4
782–783.....	1				2	1	2	6
795–796.....	1					1	1	3
806–807.....	1				1		1	3
815–816.....	1						3	4
834.....		1					1	2
Total 15	7	8	0	2	7	4	20	48
Section II								
181–183.....		1			1			2
714–716.....		1			1			2
732.....		1				1		2
733–734.....	1				1	3		5
762–763.....	2				1			3
788–789.....	1					1		2
808.....	1					1		2
812.....		1			3			4
827–828.....	1				1			2
Total 9	6	4	0	0	8	6	0	24

and like sex fraternal or unlike sex twins in the same kindred and two of these cases showed the occurrence of identical, like sex fraternal and unlike sex twins in the same kindred. On the other hand, there occurred but 9 cases where neither like sex fraternal nor unlike sex twins appeared in kindreds containing one or more identical twins. Thus it is evident that identical twins do positively occur in kindreds where like sex fraternal and unlike sex twins similarly occur and that they occur in such kindreds just as frequently and often much *more frequently* than they do in kindreds where fraternal and unlike sex twins do not occur. These conditions are shown in Table IV.

DISCUSSION

Monozygotic twins, by definition, are produced from a single zygote which, at some time during its development, divides more or less completely into two (or more) centers of active growth and differentiation. This separation is not always complete, as has been shown by Stockard (1921) for the fish, and by the numerous cases of Janus, Siamese and other twin monsters of Morrill (1919), Wilder (1904, 1908), Fisher (1866) and many others, that have occurred in man. There is still some lack of agreement concerning the exact causes of this condition, but if the work of Stockard on the fish, and of Spemann (1903) and Hey (1911) on Triton, has any transference value, it would seem that Janus, Siamese and other types of conjoined twins that occur in man are produced by the simultaneous occurrence of two separate and distinct by nearlying centers of gastrulation in the embryonic disc or blastoderm.

As development proceeds, these two centers would encroach upon each other. Certain of the most closely adjacent portions would fuse, while the remaining portions would continue an independent development. Depending upon the distance between the two centers of gastrulation would depend the degree of fusion between the two "individuals" of the twin monster, until finally

the intervening distance would become sufficient to insure the continued independent integrity of the developments from each center of gastrulation. Newman and Patterson (1910) and Patterson (1913) have described such a condition in the armadillo, while Kaestner (1898, 1907) and several others have described it in the chick, Kopsch (1895) in the European lizard, McIntosh (1868) and Reese (1911) in the cat, Carey (1917) in the pig, Bishop (1908) in the reptiles and Wetzel (1900) in the snake.

It is probable, according to Abrams (1921) and others, that all twin monsters and the most closely located, but wholly independent, twins would have but a single amnion, as in the case of the armadillo, whereas twins arising from still more distantly located centers of gastrulation might have various degrees of incompletely separated amnia until finally the condition of two distinct amnia might occur. It has been found that monoamniotic twins occur in approximately 2.11 and 2.4 per cent. of the cases of uniovular twins, according to Resinelli and Alfieri, respectively, as cited by Jeannin (1906).

The causes of this condition are, according to Stockard (1921), attributable to environmental conditions, but this view as the primary cause of twinning has been questioned by Riddle (1923), who found in his studies of identical twinning in pigeons and doves that the factors for the production of such twins operate at a stage much earlier than that of gastrulation. He was, moreover, unable to induce doubling in any of 2,500 normal eggs by means of subjecting them to various environments during or from 5 to 8 hours prior to the time of gastrulation.

It has, however, been shown that not all types of monozygotic twinning are produced by the simultaneous establishment of two centers of gastrulation in the blastoderm, for it has been shown by Assheton (1898) (1908) in the sheep and ferret, by Wetzel (1900) in the snake, by Corner (1922) and Streeter (1924) in the pig and by Streeter (1919) and Arey (1922) in man that twinning may also occur by the simultaneous development of two inner cell

masses in mammals or by the development of two or more separate and distinct blastoderms in reptiles. Corner (1922) attempts to correlate these two general types of twinning by suggesting that there might be intermediate and unclassified stages. The two inner cell masses of the latter type of twinning may be separated by a varying distance, and may, on the one extreme, come to lie so closely together that the two blastoderms of the two inner cell masses fuse into one, but each develops its embryo independently as though the fusion had not occurred.

And finally, it seems, if the work of Driesch (1892) with the sea urchin, Wilson (1893) and Cerfontaine (1906–1907) with Amphioxus, Spemann (1914) and Rudd (1925) with Triton, Morgan (1893) with the fish, McClendon (1910), Schultz (1894), Morgan (1895) with the frog, and many others, in which it was found that two embryos, or twins, could be produced by the separation of the two primary blastomeres, has transference value when applied to man, then a third type of monozygotic twinning might be expected. Twins of this type, though monozygotic, would be expected to have separate chorions and placentae. The frequent difference noted by Danforth (1916), Curtius (1928, 1930), Lassen (1931), and several others, between the number of monozygotic twins when determined by the "difference method" and by the "similarity method," on the one hand, and by the "birth membrane method," on the other, might in part be considered to represent monozygotic twins of this type.

Dizygotic twins, by definition, are produced from two zygotes that arise and become successfully implanted during the same oestral cycle. It has been shown by Arnold (1921) and Davenport (1920) that probably between 5 and 10 per cent. of all ovulations are double. Ova, therefore, seem to occur in an excess of about 4 to 9 times the frequency of twin births. Davenport considers this to indicate that the limiting factor for the production of dizygotic twins lies elsewhere than in the number of ova available for fertilization during a single ovarian cycle.

These limiting factors may reside in some phase of the fertilization process, in lethal factors of some sort occurring in the zygote, in the implantation mechanism, or in subsequent early incompatibility between mother and embryo. Davenport seems to emphasize the importance of lethal factors introduced into the zygote by the paternal or the maternal line.[2]

The relation of these varying modes for the development of monozygotic and dizygotic twins to fundamental, underlying genetic factors may be of several sorts. It has been shown by Morgan, Sturtevant, Muller and Bridges (1923, pp. 32–34) that two or more phenotypic characters may always occur together. Such cases are considered to be so closely linked that they have a crossover value of zero, hence are considered each to have exactly the same gene locus, or, more simply, to be two manifestations in different portions of the phenotype of one and the same gene. There could be no more reason for suspecting, *a priori,* that such phenotypic characters might be thus related than for suspecting, *a priori,* that two phenotypic characters might be genetically linked. Such occurrences are detected entirely by genetic precedures, and proof of their occurrences rests entirely on such determinations.

[2] A third type of twinning has been suggested by Danforth (1916), Fisher (1919) and Curtius (1927), and been regarded favorably by several others. The general idea involves the formation of two cells, each containing a female pronucleus from the original ovum. This may occur either, as Danforth suggests, by a precocious division caused by the penetration of the egg by the spermatozoon so that the male pronucleus is able to unite with only one half of the original egg nucleus, leaving the other half free for fertilization by a second spermatozoon, or as Curtius suggests, by the fertilization by separate spermatozoa of both the definitive ovum and its unusually large second polar body. In view of the lack of any embryological evidence concerning the occurrence of this type of twinning, one is forced to regard it skeptically, despite the fact that it would seem to explain many of the difficulties of Fisher (1919) and others who, from statistical studies on twins classified by means of the "similarities method," find need for a type of twin that is genetically intermediate between the completely dizygotic twin with its four different pronuclei and the monozygotic twin with only two different pronuclei.

It may be, however, that these types of twinning are produced by one of these two processes. If subsequent studies show that all types do always occur together in the same kindred, then depending upon the nature of the key process concerned respectively in the production of each type, and the results of genetic studies in order to test linkage, it might be found that these types of twinning are either the expression of several different and entirely distinct phenotypic manifestations of the same gene, or are the phenotypic manifestations of several genes which, however, are linked with a to-be-determined degree of closeness.

On the other hand, it has been shown by Hoge (1915) and others that a gene may produce a phenotypic character or condition which of itself may underlie or lead to the production of numerous phenotypic characteristics, the origin of all of which, however, may be traced eventually to the one genetically determined phenotypical condition. *A priori,* one would expect to be able to detect such a condition by noting some sort of fundamental similarity among several phenotypical characteristics that seem to be related and also genetically determined. Intergradations between the two types might very conceivably occur.

From this point of view, it seems reasonable, on the basis of the foregoing discussion and on the basis of the observations presented in this article, to suggest that possibly a single genetic factor might be responsible for the production of a fundamental underlying phenotypic condition which might secondarily produce or manifest itself in a highly varied but always fundamentally similar manner. Such a character in these cases of twinning might be termed "*Weak Dominance,*" and might be specifically defined as "a tendency, manifested throughout the early life of the zygote, for primary growing points to fail to adequately dominate the secondary and other potential growth areas, with the result that, frequently, two primary growing points arise where normally but one would occur."

In the case of monozygotic twinning of the type where the two blastomeres separate, this might take the form of a weakened dominance of the organism as a whole over its constituent parts. The work of F. R. Lillie (1902, 1906) on the differentiation of the egg of *Chaetopterus* with cleavage experimentally suppressed would indicate that what might be termed organism-as-a-whole forces are, in this case, independent of the cleavage mechanism. If such experiments have transference value, it might indicate that similar conditions might occur in man. On the other hand, the work of Herbst (1900), on raising eggs of echinoderms in calcium free sea-water would indicate that separation of the blastomeres is largely a process dependent upon environmental influences.

The manner of formation of the inner cell mass may also be one of dominance. It would seem from the observations of Assheton (1898), Corner (1922) and Streeter (1924) that at least more than one group of the cells of the morula can form an inner cell mass, or that the cohesive forces which ordinarily cause all the cells of the morula to remain together may occasionally be weakened so as to lead to a splitting whereby these inner cells form two separate and distinct masses, each of which undergoes subsequent independent development. In either of these two methods, a slight weakening of the normal dominance of one part over the other parts at a certain time may very readily lead to the establishment of two equally strong growth points instead of the one that normally occurs.

The importance of dominance by primary growth centers in the blastoderm at the time of gastrulation, and the frequent production of twinning when such dominance is disturbed at certain times by experimental methods has been extensively described by Stockard (1921). Any weakening of the dominance of the primary centers of gastrulation at this stage produced by hereditary factors might, likewise, very possibly, result in the establishment

of two centers of gastrulation instead of one as occurs normally.

And finally, in the case of the dizygotic twinning, from this point of view, the underlying cause might reside in a weakening of the dominance of the zygote at the time of implantation in such a way that the uterus would remain receptive for a longer time than normally. In such a case, it might frequently happen that a second zygote might become well implanted before the uterus would be rendered utterly unreceptive. Certainly, it has been shown by Leo Loeb (1923), Corner (1921), Courrier and Kehl (1930), Knaus (1930), Asdell (1928), Pratt (1927) and several others that the effect of the implantation of the zygote is to cause the retention of the corpus luteum, which in turn secretes hormonal substances which render the uterus retentive of zygotes already implanted, but unreceptive to others.

The nature of the dominance mechanism is still subject to some question, but the work of Jacques Loeb (1924) on Bryophyllum and of Spemann (1936) on "organizing substances" in the dorsal lip of the blastopore indicate that definite substances are involved. It is conceivable that the amounts of such substances might vary and that the amounts might be determined by underlying hereditary factors.

The association between the occurrences of identical and fraternal twins demonstrated in this paper confirms the observations of Curtius (1927) and Dahlberg (1930). Curtius, relying solely upon identifications on the basis of physical similarities, notes cases of the occurrence of like sex fraternal and identical twins in the same families and sometimes in the same fraternities, and cites in addition some cases of Battstrom (1914) and Hoehne (1920) of triplets, quadruplets and quintuplets in which some of the individuals are clearly identical and some clearly fraternal. His material, however, is somewhat limited.

Dahlberg (1930) provides some data obtained from mothers who had already given birth to one pair of twins.

Not counting these twins, he obtains a ratio of 48 like sex pairs to 24 unlike sex pairs from mothers who had previously produced an unlike sex pair of twins, and again not counting the first twins, obtained a ratio of 18 unlike to 38 like sex pairs from mothers who had previously produced a like sex pair of twins. It seems as though the exclusion of the first pairs of twins born to these mothers significantly reduces the value of the conclusions which he derives from these data.

Working with other forms, Green (1934) has explained certain genetic ratios which he obtained from crosses of mice on the basis of the occurrence of hereditary monozygotic twinning, while Danforth (1925) by selecting parents who had produced incompletely separated twin monsters was able to isolate strains of mice having a markedly augmented incidence for the production of such twins. The cases where monozygotic twins have been described in the pig by Corner (1922) and Streeter (1924) show that in that form identical and what would correspond to fraternal twinning occur together, though the origin of the material rendered impossible studies for the determination of the mode of inheritance of such twinning.

On the other hand, Bonnevie (1925) and Greulich (1934) have definitely concluded from their observations that dizygotic and monozygotic twinning are quite distinct from each other. Bonnevie's conclusion follows directly from her observations that only the dizygotic type of twinning is hereditary, but Dahlberg (1930) has computed the probable errors for her data and finds that the amount of her data concerning one-egg twinning is much too small to allow of reliable conclusions.

Greulich, basing his conclusions on a population of 312 individuals containing 94 monozygotic twins in 91 families of sizes from 1 to 10 births and on a population of 1,017 individuals containing 296 twins in 270 families of sizes from 1 to 10 births, concludes that "the parents of monozygotic twins differed from the parents of dizygotic twins

in their capacity for twin production: the latter had twins much more frequently than would be expected if twinning were determined by chance alone, while the former showed a frequency of twin births only very slightly higher than chance expectation." In view of the fact that he computed the incidence of monozygotic twins in the general population to be approximately 4 per 1,000, and obtained 3 per 221 individuals, and for dizygotic twins to be approximately 7.5 per 1,000, and obtained 26 per 747 individuals, it would seem that the sizes of the populations that he used for these determinations were much too small to justify his conclusions. It would be interesting to know the distribution of monozygotic and dizygotic twinning in his studies of the incidence of twinning among the sibs of twin-producing parents.

It has been shown in this article that the factors for the production of the two types of twinning occur together with a significantly high degree of regularity. The causes of this association may be any one or combination of several conceivable possible factors. Twin production is a very complex phenomenon that requires for its successful completion an optimal phase of a wide variety of fundamental and underlying, contributory conditions of the nature of high general gametic compatibility and vitality, anatomical suitability, nutritional and physiological vigor and generally favorable environmental conditions.

In so far, however, as twinning may be proved to rest upon a distinctly genetic basis, so then this demonstration of the association between the two types of twinning might be regarded as an indication of a genetic linkage *of some sort* between the factors for the production of identical twins and the factors for the production of fraternal twins. If further studies should prove that the determining factors are dependent upon genetically linked genes, then one would have good reason to expect to be able to discern the occurrence of autosomal linkage in these phenomena.

SUMMARY

An association between the factors for the production of identical and those for the production of fraternal twinning has been shown to occur rather widely and in a significantly large majority of the kindreds, irrespective of their size. The occurrences of this condition have been shown both by means of the "difference method" of analysis for the ratios of like and unlike sex twins that occur in the same kindred, and by means of a detailed study of the occurrences of specifically described identical twins in kindreds which also contain specifically described like sex fraternal or unlike sex twins. It was demonstrated that identical twins do positively occur in such kindreds and that they occur in that way much more frequently than they do in any of the other kindreds. It has been shown conclusively that the chance coincidence of small groups of identical and fraternal twins in the same kindred certainly could not occur frequently enough to account for the distributions which have been found.

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ORDER OF DIFFERENTIATION IN RELATION TO ORDER OF DETERMINATION IN GAMIC FEMALE APHIDS

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INTRODUCTION

WHEN winged parthenogenetic female aphids in the gamic phase of the cycle are first reared in low temperatures and then changed to high temperatures, their successive offspring gradually change from gamic to parthenogenetic females (Shull, 1930a). Aphids intermediate between gamic and parthenogenetic are born during the transition. Studies on the composition of the intermediate aphids (Shull, 1930b, 1931, 1933) have shown that the first intermediates to appear have modified antennae and hind tibiae, later offspring showing changes in the reproductive system. In these modifications of the reproductive system the earliest offspring lose their colleterial glands and seminal receptacle, while later ones exhibit various changes in the ovarioles. The order of change is thus antennae and hind tibiae, then colleterial glands and seminal receptacle, then ovarioles.

A possible explanation of this order of change in the intermediates (Shull, 1930b), similar to that used by Goldschmidt (1927) to explain intersexes, is that only those structures in the embryo are affected that have not been determined at the time of the temperature change. Thus the first structures to be changed (antennae and hind tibiae) in a series of successive offspring would be those that are determined late, the last to be changed (ovarioles) in such a series would be those that are determined early. The order of embryonic determination thus would be ovarioles, then colleterial glands and seminal receptacle, then antennae and hind tibiae.

If determination of a structure leads to its differentiation soon thereafter, or if equal periods of time intervene

between determination and differentiation, the differentiation should likewise be (1) ovarioles, (2) colleterial glands and seminal receptacle, (3) antennae and hind tibiae. It is important to know whether this is the order. This paper gives the results of an investigation designed to determine the order and time of differentiation.

Material and Methods

The order of differentiation was established by the study of slide preparations of gamic embryos and first, second and third instar gamic females. Both parthenogenetic and gamic females were used for the study of time of development of the different structures. As all aphids with the exception of the stem mother are produced viviparously and parthenogenetically there is no externally observable event such as fertilization or egg deposition by which the beginning of development can be timed. It was observed, however, that parthenogenetic embryos about to be born contain within their ovarioles embryos of the next generation. The oldest of these embryos are well along in cleavage when the parent aphid is born. The development of the newly born aphid into an adult is paralleled by the development of the enclosed embryos so that the time elapsed from late cleavage to the development of any particular structure in the oldest embryo can be determined by fixing the parent aphid at a measured time after birth and examining the oldest enclosed embryo. To determine the total age of the oldest enclosed embryo at any particular time it is necessary to add to the above time the time elapsed between maturation of the parthenogenetic egg and late cleavage. It is impossible to determine the beginning of maturation without killing the egg and thus stopping development, hence the total time of development can not be computed for any one aphid. This can be done only by adding the time of development of the two different stages taken from two different aphids. As variation occurs in the rate of development in different individuals an accurate measure can be made only by

statistical handling of a great number of individuals. Sufficient specimens were not collected to do this, hence all references to time of development refer to time measured from late cleavage.

Measurements of time of embryonic development computed indirectly on the basis of rate of growth of a maturing aphid are subject to variation. Shull (1930a) has shown that the rate of postnatal development of aphids is modified by the prenatal conditions of light and temperature. By varying the light and temperature conditions applied to the parents he found that some parthenogenetic females matured at the mean age in days of 8.0 ± 0.10, while others did not mature until reaching a mean age in days of 12.0 ± 0.91. My own observations indicate that a variation in the rate of postnatal development occurs under ordinary laboratory conditions. Of 17 cases recorded one required 6 days to become adult while several required 9.5 days. The mean age in days of the 17 aphids was $8.2 \pm .26$. That this variation of postnatal development has an effect on the rate of development of the embryos contained within the growing aphids is shown by a comparison of a fourth instar aphid with an adult, both of which were 8 days old. The embryos in the adult were much more advanced in development than those in the fourth instar.

It is possible that the variation in rate of embryonic development caused by a variation in rate of postnatal development of the parent affects certain periods of embryonic development only and does not affect the total time of development, which thus may be the same for all aphids. A microscopic examination and comparison of embryos of the same age would show a difference in degree of development if such exists, but would give no indication whether these differences were equalized by the time of birth. It is possible that retardation of development at one stage would be compensated for by later increase in the rate of development and, conversely, that an early rapid rate of development would be equalized by a later slow rate.

The age of parthenogenetic aphids at the last molt varies in 17 observed cases from 6 days to 9.5 days. At the time of birth each of the 17 aphids contained one or more embryos undergoing cleavage, which at the time of the last molt would be almost completely developed and ready for birth. The age of the embryos would vary depending upon the age of the parent so that the oldest embryos contained in the aphid that matured at 6 days of age would be 6 days, while the embryos contained in the aphid that matured at 9.5 days would be 9.5 days old. If the total time of embryonic development is the same for all aphids the time between the last molt and the birth of the first young should be at least 3.5 days longer for the former aphid (6 days) than for the latter (9.5 days). Such a difference in time between the last molt and birth of the first young does not exist. The aphid that matured at 6 days of age gave birth to its first young 24 hours later, and one of the aphids that matured at 9.5 days likewise produced its first young 24 hours later. Thus the total time taken for embryonic development was 7 days in one case and 10.5 days in the other. One aphid that matured at 9.5 days produced its first offspring 46 hours after the last molt, thus making the total time of development for this embryo approximately 11.5 days.

ORDER AND TIME OF DIFFERENTIATION OF REPRODUCTIVE STRUCTURES

The first reproductive structures to appear in the embryo are the germ cells. No attempt was made to discover their exact origin, but by the time the blastoderm is formed about 24 hours after early cleavage the germ cells are evident as a rounded cluster of cells within the blastocoele (Fig. 1). At this time the blastocoele is being filled up with small corpuscles that later make up the symbiotic organ. These corpuscles are derived directly from a localized area on the ovariole containing the developing embryo. The germ cells lie at one side of the blastocoele, hence are not quite surrounded by the newly formed

symbiotic organ. This same spatial relationship between germ cells and symbiotic organ is continued throughout the life of the aphid. The germ cells increase in number as development proceeds and become arranged in clusters, each of which is surrounded by a thin membranous sac. Five days after cleavage the clusters of germ cells are recognizable as germaria in which the nurse cells and oogonia have become differentiated. By this time the body cavity is formed and the germaria are found ar-

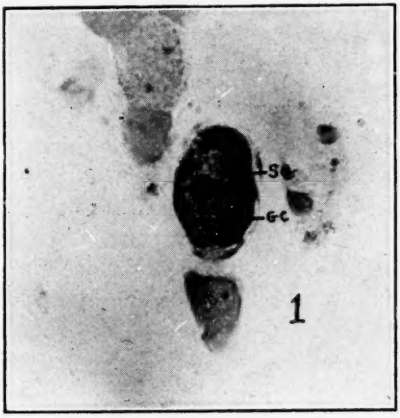

FIG. 1. G C—Germ Cells; S O—Symbiotic Organ.

ranged in two groups one on each side of the cavity, the rest of which is filled with the symbiotic organ.

Ovarioles appear about 5 days after cleavage as outgrowths of the membranous sacs surrounding the germ cells. A short distance from the germaria the ovarioles of each group unite forming two parallel oviducts which extend to the posterior lateral tip of the abdomen. The end of each oviduct enlarges into a terminal ampulla, the base of which rests against the thickened ectoderm ventrad and laterad of the anus. Thus one terminal ampulla is right and the other is left of the ectoderm immediately ventrad of the anus. The oviducts develop on the sixth day after cleavage.

It is important to know at what time during development the ovarioles first exhibit their gamic nature. For at least 24 hours after the ovarioles are differentiated there is no visible difference between gamic and parthenogenetic types. Some time before birth (about 24 hours) parthenogenetic ovarioles receive eggs undergoing maturation. Ovarioles of gamic females of the same age are devoid of eggs and do not contain eggs until 4 or 5 days after birth. This lack of reproductive activity in embryonic gamic germaria and ovarioles in contrast to the production of eggs in the parthenogenetic females indicates that determination of both types has occurred within the embryo.

Gamic eggs probably begin maturation during the fourth instar. The evidence is not exact on this point, but none of the slides of third instar gamic females show maturing eggs, while all the adults show the eggs plainly. There is some doubt that the slides of fourth instar aphids are actually fourth instar, hence the evidence from these slides is omitted. The inability to state the exact time of maturation of gamic eggs is of no great importance at this time. The significant fact is that gamic eggs are produced after birth only. This point will be enlarged upon later.

The vagina is differentiated next as an invagination of the ectoderm ventrad of the anus and between the two terminal ampullae. The first indication of the development of the vagina is a thickening of the ectoderm between the terminal ampullae (Fig. 2). This occurs about the seventh day after cleavage or approximately 48 hours before birth. The invagination begins about 24 hours later and continues after birth.

The colleterial glands and seminal receptacle develop as outgrowths of the vagina during the second instar (2 or 3 days after birth and 5 or 6 days after differentiation of the ovarioles). One colleterial gland appears on each lateral side of the vagina while the seminal receptacle develops as an evagination of the dorsal vaginal wall (Fig. 3).

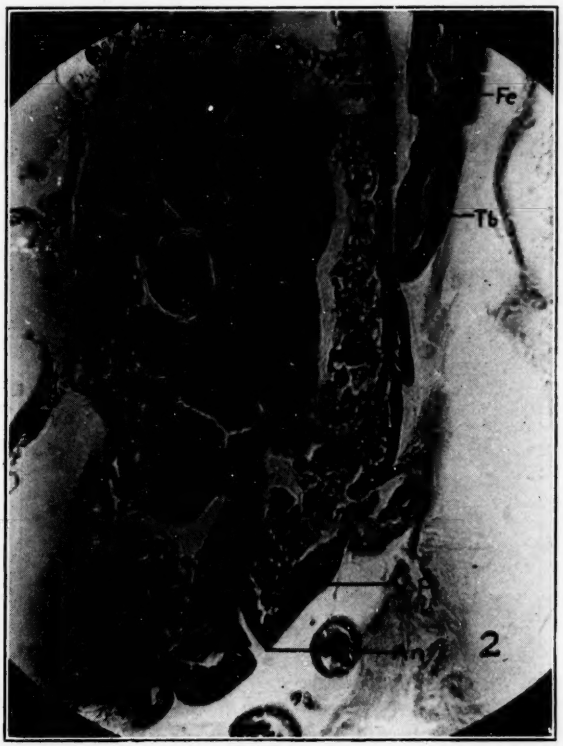

FIG. 2. An—Anus; Fe—Femur; Tb—Tibia; V P—Vaginal Primordium.

It has been shown by Shull (1930a) that determination of the colleterial glands and seminal receptacle occurs before birth. Hence the appearance of these structures 2 or 3 days after birth indicates a delay in differentiation. Evidence of an earlier differentiation within the embryonic period was sought by comparing the vaginas of gamic and parthenogenetic female embryos. Any character by means of which gamic and parthenogenetic female embryonic vaginas could be differentiated could be used in the case of the gamic female to predict the future appearance of the colleterial glands and seminal receptacle, and consequently the appearance of this vaginal character could be

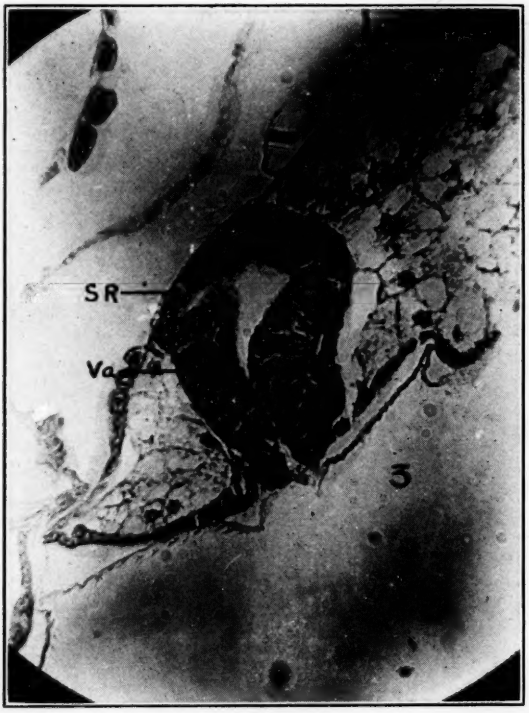

FIG. 3. S R—Seminal Receptacle; Va—Vagina.

considered to be the first indication of the differentiation of the colleterial glands and seminal receptacle. No such character was found so it must be concluded that a delay in differentiation actually takes place.

DIFFERENTIATION OF HIND TIBIAE

The appendages begin development as outgrowths of the sides of the embryo on the fourth day after cleavage (Fig. 4). Twenty-four to 48 hours before birth they differentiate into femur, tibia, tarsus and claws. Fig. 2 shows a longitudinal section through the joint connecting the femur and tibia of the metathoracic leg shortly after segmentation of the appendage. Further development

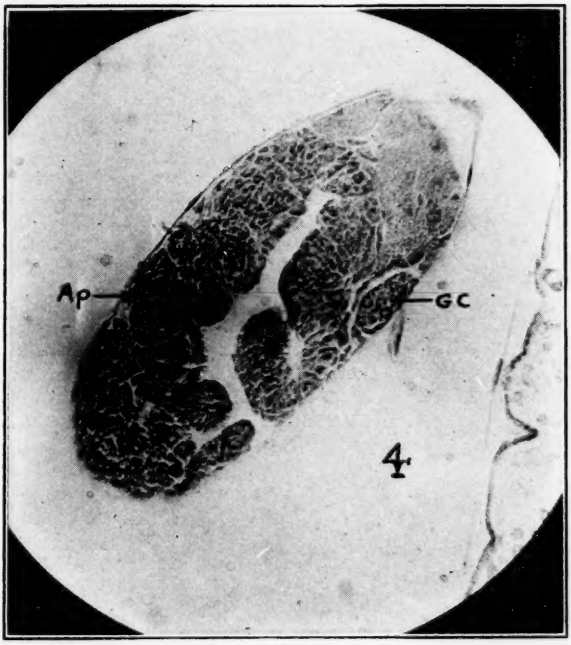

FIG. 4. Ap—Appendage; G C—Germ Cells.

consists mainly of growth in size until at the time of the last molt (8.2 ± .26 days after birth) when the hind tibiae undergo their last change and develop gamic characters which consist of a swollen condition, dark coloration and sensoria (Fig. 5).

It is questionable which event in the development of the hind tibiae represents the most significant period in relation to the determination of the gamic nature of the appendage. Differentiation of the hind tibiae undoubtedly occurs at the time of the segmentation of the appendages. However, as there is no morphological difference between parthenogenetic and gamic female appendages at this time there is no reason for assuming that this event constitutes the differentiation of the gamic nature of the hind tibiae. The appearance of the adult gamic hind

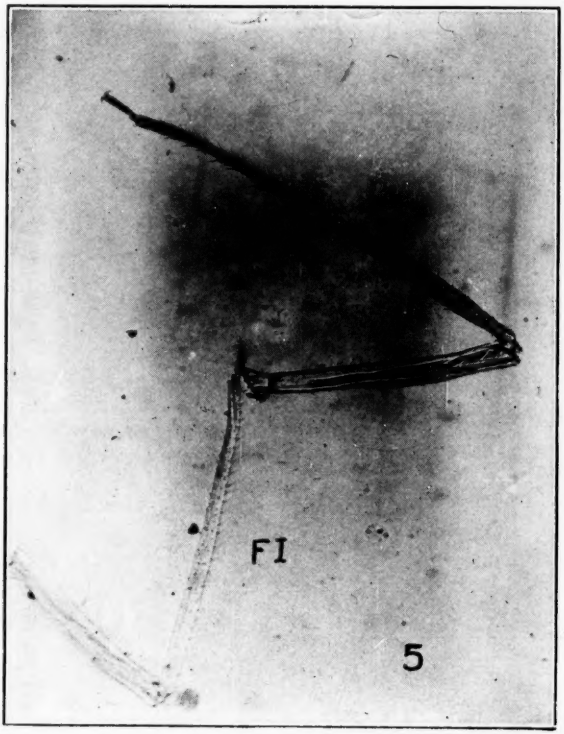

FIG. 5. Ad—Adult; F I—Fourth Instar.

tibial characters at the time of the last molt marks the first visible difference between gamic and parthenogenetic hind tibiae, consequently this event is assumed to be the differentiation of gamic hind tibiae. It is probable that some sort of physiological differentiation occurs before this, especially since determination occurs before birth, and that the gamic hind tibial characters simply represent the adult expression of the earlier differentiation. Despite this probability it is necessary to conclude on the basis of the visible evidence that differentiation does not occur until the last molt and, therefore, that differentiation is delayed about 8 days after determination has taken place.

Order of Differentiation Compared to Order of Determination

The order of differentiation is (1) ovarioles, which appear about the fifth day after cleavage, (2) colleterial glands and seminal receptacle, which appear two or three days after birth, (3) hind tibiae upon which gamic characters develop about eight days after birth. Proof that the order of differentiation is as stated is given in the illustrations. Fig. 5 shows a fourth instar and an adult hind tibia. The absence of swelling and sensoria on the fourth instar appendage is apparent. In Fig. 3 the seminal receptacle is shown in the early stage of its growth from the dorsal wall of the vagina. This figure is to be compared

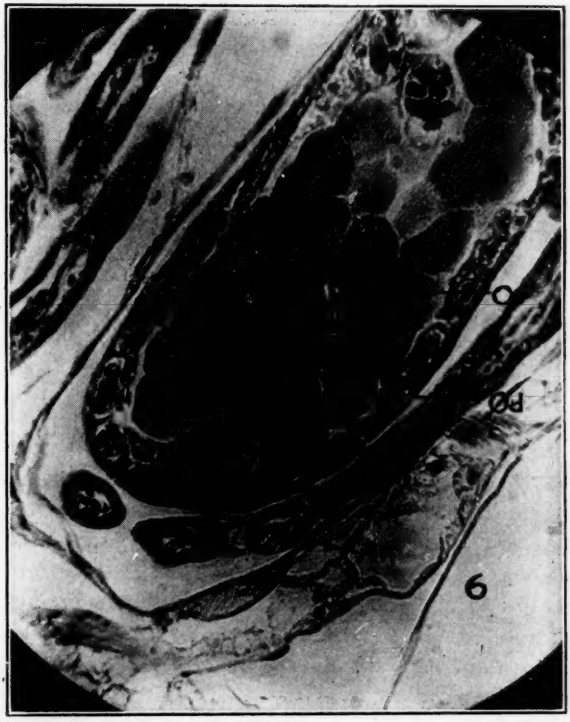

Fig. 6. An—Anus; Od—Oviduct; Ov—Ovariole.

with Fig. 6, which shows no evidence of a vagina but which does show the presence of ovarioles and an oviduct. The evidence from intermediate aphids that determination occurs in the following order (1) ovarioles (2) colleterial glands and seminal receptacle (3) hind tibiae is thus supported by the order of differentiation.

DISCUSSION

Certain facts developing out of the study of differentiation indicate that the original hypothesis which explains the production of intermediate aphids on the basis of different times of determination for different structures may be partially correct only. The time of determination theory explains the production of intermediates by assuming that in any embryo the structures involved have two possible directions of development; for instance, ovarioles may be either gamic or parthenogenetic. The factor which determines which direction the structure will take in its development is the concentration of a substance, let us say, in the embryo at the time that determination of the structure takes place. If this concentration is high, development will be in one direction; if it is low, development will proceed in the other direction. After determination no change can take place in the direction of development.

Shull (1930b) describes one type of intermediate aphid which contains a gamic egg at the base of one ovariole with parthenogenetic eggs and embryos beyond. The presence of the gamic egg in the ovariole necessitates a gamic germarium at the end of the ovariole, while parthenogenetic eggs and embryos in the ovariole require that the ovariole be of the parthenogenetic type. Unless there are two germaria attached to one ovariole, one gamic and one parthenogenetic that produce eggs in that order it is necessary to conclude that the original germarium was gamic and that it was changed to the parthenogenetic type. Shull does not state which type of germarium was present when he observed the ovariole.

In the strain of aphids used in the study of differentia-

tion gamic eggs are produced by the germarium after the third instar only. If this is true also of the strain used by Shull at least one germarium of the above intermediate must have been altered several days after birth, and consequently after embryonic segregation. The above intermediate may be an unusual exception; nevertheless, it indicates that structures once determined might be changed in their nature and that such changes may occur after birth.

Other intermediates described by Shull (1930b) supplement the evidence that modification of structures after birth is possible. These intermediates are characterized by the following types of structures: (1) eggs which are partly gamic and partly parthenogenetic, (2) colleterial glands reduced in size, (3) hind tibiae partly swollen.

As all these intermediates were produced during a change from gamic to parthenogenetic females it is possible that the intermediate eggs were produced first as gamic eggs and later changed toward the parthenogenetic type. The only time such a change could occur is after the third instar, as gamic eggs are not produced until this time. There remains the possibility that the eggs were intermediate at the time they passed from the germarium into the ovariole. The germarium in this case would be probably intermediate also. This would exclude the necessity of a change after birth as parthenogenetic eggs are produced during the embryonic period and it is conceivable that intermediate eggs could be produced at the same time.

The intermediates with partly developed colleterial glands and hind tibiae offer somewhat weightier evidence that the combination of factors that cause intermediates is active during postnatal development. Both the colleterial glands and hind tibiae are differentiated after birth, hence whatever inhibited their full development in these intermediates must have been operative at the same time.

The recent analysis of intermediate-winged aphids by Shull (1937) indicates that in this type of intermediate the

order of determination is not the only cause of the production of intermediates.

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SHORTER ARTICLES AND DISCUSSION

FRESHWATER JELLYFISH RECORDS SINCE 1932

As so many isolated and scattered references to freshwater jellyfish have appeared in recent years, I have assembled for ready reference all American records, both published and unpublished, that have come to my attention since Dejdar's (1934) monographic treatise, "The Freshwater Medusa, *Craspedacusta sowerbii.*" His list carries the known records through 1932. The last American entries are those of Kraatz (1933) for Ohio, and Brooks (1932) for Pennsylvania.

Dejdar appears to have inferred (p. 674) that the slow sand filters of the Washington, D. C., filtration plant from which I reported *Craspedacusta* in 1927 (1927) lay close to Great Falls, Md., from above which the city's water supply is taken. These particular filter beds, in truth, lie at a distance of 17.1 miles by underground aqueduct from Great Falls. There are two interruptions to the direct flow of the water, the first at the Dalecarlia Reservoir, 9.5 miles from Great Falls, and the second at the McMillan reservoir, 7.6 miles by aqueduct from Dalecarlia. The water is drawn directly from this second reservoir for filtering at the filtration plant located at Michigan Avenue and First Street, N.W. The Dalecarlia reservoir lies in a natural valley and receives some surface drainage from a well-protected area; the McMillan reservoir, however, is surrounded on all sides by ample concrete gutters and receives very little if any surface water.

No jellyfish have ever been observed in the Washington water supply system since the original and unique find of 1927. But Mr. J. W. Keys, who first discovered medusae in a "pot-hole" along the river shore just above Great Falls that year, secured additional specimens two years later, August 18, 1929, from the same pot-hole. These he presented to the National Museum. Miss Lillian Cash and Miss Edith R. Keleher, who each fished a number of specimens from Black Pond, Va., on August 11 and 27, 1929, respectively, also turned them over to the museum. This pond lies about one and a half miles below Great Falls and just below the mouth of Difficult Run. The pond is separated from the run by a rocky cliff. Lying about a hundred yards or so from a bend in the river, Black Pond at extreme high water is on occasion overflowed by the river.

In September of 1930 we determined a specimen of *Craspedacusta sowerbii* for Professor P. C. Bibbee, of the Concord State Normal School, at Athens, W. Va. This he had collected in West Virginia, presumably near Athens, in Mercer County, in the southern, mountainous part of the state. Athens lies at an elevation of 2,596 feet. It is a first record for the state.

About mid-August, 1931, Mr. Stanley G. Jewett, of the U. S. Biological Survey, stationed at Portland, Oregon, sent the National Museum seven specimens of *Craspedacusta sowerbii* (kindly determined for us by Dr. H. B. Bigelow) which he had obtained from the Willamette River, near Portland (Portland Harbor). In a recent letter he writes that the jellyfish seem never to have reappeared in the river. He took pains to inquire of friends who could be expected to have had knowledge of any recurrence of them. This is a first record of the occurrence of the freshwater jellyfish under natural conditions on the west coast.

On September 20, 1932, Mr. G. L. Hackleman, of Vandalia, Ill., sent in a sketch of a freshwater jellyfish, an undoubted *C. sowerbii*, which he had observed in numbers in a rock pool on the grounds of his Old Capitol Nursery Co. Van Cleve (1936), however, antedates this Illinois record by specimens found in a concrete garden pool in the same city by Mr. D. J. O'Donnel. The medusae were first noticed in 1931 and, although the pool was drained that winter, they were again seen on September 5, 1932, but disappeared by the 20th of the month. Van Cleve mentions also the discovery of jellyfish in a concrete fish and lily pond at Carmi, Ill., by Mr. John Cralley. These endured from August 8 to September 20, 1933, but failed to return in either of the next two summers, although a close watch was kept for them.

Allyn and Rettgar (1933) found large numbers of medusae of *Craspedacusta* in an old gravel pit three miles north of Terre Haute, Ind., first on August 20, 1932. The animals continued through August and some indeed persisted as late as October 10. This body of water is "totally shut off from the Wabash River, and received its water by drainage from the adjacent land. It is approximately 100 yards wide and one fourth of a mile long. So far as known, it has never been stocked with fish, but is used to a great extent by water birds during migration." Although no definite record was kept of the reappearance of these medusae in this gravel pit, Dr. Allyn writes me that they have been noted on three different occasions since 1932, that either they did not

appear every year or else escaped notice at one time or another. However, they were found in this gravel pit again in 1937.

In July, 1933, considerable numbers of jellyfish appeared in a goldfish pond in the garden of the Reverend William B. McIlwaine, of Alexandria, Va. The water supplying this pool comes from the local water mains. I have just learned from Mr. McIlwaine that the jellyfish appeared only the one year in his pool.

About this same time, Professor J. W. Bailey, of the University of Richmond, brought me several of these medusae from an old quarry pool near that city. In a recent letter he states that they were also found in 1935 and 1936 in three different quarry pools several miles apart, and again in 1937, but apparently not in 1934 unless they escaped notice.

Kraatz in 1933 reviewed the Ohio records that had been published at an earlier date by Baird (1932) and noticed by Dejdar. This same year, also, Woodhead (1933) found jellyfish in the Huron River near Ann Arbor, Mich.

The next year, 1934, Mrs. Imogene Robertson (1934) reported the appearance of freshwater jellyfish in a pond a few hundred feet from Lake Erie at Bay View, Lackawanna, a suburb of Buffalo, N. Y. From Texas Cheatum (1934) reported, July 25, 1934, literally thousands from a small artificial pond located near the city limits of Dallas. A number of specimens from this pond were collected by Dr. C. E. Burt that summer for the National Museum.

Two years later, October, 1936, Mr. Alfred C. Weed, of the Field Museum in Chicago, informed me that Mrs. William Pappas, of San Antonio, Texas, had been having a great deal of trouble with freshwater medusae in her garden pool that summer. They seemed to be present at almost all seasons, but became very numerous in warm weather. Mrs. Pappas told Mr. Weed that the jellyfish did a great deal of damage to the goldfish which were kept in the pool, that apparently whenever one touched the tail of a fish it seemed to cause such an injury that a portion of the fin dropped off. This is the first instance of which I have ever heard in which these jellyfish had caused injury. In compliance with a request made at that time for specimens, Mrs. Pappas forwarded several preserved in formalin to the museum the following summer, stating that the jellyfish had reappeared in her pool on July 27, 1937.

Harbaugh (1937) established a first record for Kansas when he reported the presence of a number of specimens in a private fish pool in the town of Manhattan during the month of July, 1936.

Iowa's first record was made the same year, when Mr. W. W. Aitken (1936), of the State Conservation Commission, found the species in a gravel pit called Avon Lake, ten miles southeast of Des Moines. They lasted here for a month, from August 20 to September 20, 1936. It was this same fall that Mr. Arthur W. Weidner, of Gettysburg College, Gettysburg, Pa., wrote the National Museum that he had found thousands of jellyfish in an old quarry close to town. This excavation had originally been made for clay, but was now filled with water. Mr. Weidner writes me that, although he watched the water in this quarry daily during the months of September, October and November, 1937, the jellyfish did not reappear.

On August 20, 1936, Atwood and Steyermark (1937) collected several medusae from a rock-margined pool along a "shut-in" of Marble Creek, near Fredricktown, Mo.

In a very recent summary, Breder (1937) lists several occurrences that have received little or no published attention: (1) "an outbreak of jellyfish in the water supply of Birmingham, Ala., during late July and early August of 1933," and again the following year; (2) Brady's Pond, Staten Island, where Mr. William T. Davis (1937) discovered freshwater jellyfish the first time in 1933 and where they have been seen again each year since that time, including 1937, a very consistent record, in view of the otherwise apparently sporadic and irregular occurrence of the animals; (3) Mr. Davis's (1937) report that jellyfish had also been found in Cranberry Lake, Sussex County, N. J., on August 29, 1937, by Miss Jane De Puy; (4) Franklin, Ind., where some fishermen found medusae in an old gravel pit near Sugar Creek, about seven miles east of Franklin, late in September, 1931. Dr. Naomi Mullendore, of Franklin College, who informed Breder of this discovery, added that no medusae were found the following year, or in 1936 or 1937, and no hydroids at any time.

On August 16, 1937, Dr. Dayton Stoner, of the New York State Museum, had his attention called to the occurrence of *Craspedacusta* in a fish pond at Loudonville, N. Y., three miles north of Albany. Notes upon the discovery and some observations upon the animals themselves have recently been published (1938).

On August 24, 1937, the National Museum received a number of

specimens from Dr. Kimber C. Kuster, of the Bloomsburg, Pa., State Teachers College. They were found in an abandoned limestone quarry filled with spring and surface water. This quarry is located about four miles east of Bloomsburg, near Almedia, Columbia County, and about one-half mile north of the Susquehanna River, at an elevation of 480 feet above sea level and perhaps 30 feet above the river. Medusae were collected in the quarry pool. They also developed in a laboratory aquarium containing water and water weed, *Elodea,* brought from the pool. Dr. Kuster has submitted a note regarding this find to *Science* for publication (1938).

In *Science* for November 26, 1937, Hamaker and Milne (1937), of Randolph Macon Women's College, report *Craspedacusta* from Crystal Lake, 14 miles south of Lynchburg, Va., while in the December 17th issue, Quick and Matthews (1937), state that freshwater jellyfish taken in Sandy Lake, Stoneboro County, Pa., by John Hines in 1936 were again found in the same body of water in 1937.

Under the date of January 25, 1938, I received a most interesting letter from Miss Eloise Kuntz, of the University of Washington, who tells me that last November she "found medusae in the sixteen tentacle stage in her twenty-gallon aquarium. They were common during the whole month of November, and were seen in small numbers during December." During that month two tropical fish were added. On January 20 a few medusae were still to be seen. None developed beyond the sixteen tentacle stage. She also found hydranths entangled in the algae and debris on the glass sides of the tank. As early as 1927, writes Miss Kuntz, Professor Kincaid observed large numbers of very small, immature medusae in a tank in the University Department of Fisheries, where they persisted for about two weeks. Dr. J. E. Lynch, of the Department of Fisheries, informed Miss Kuntz that hydranths were present in one of their tanks all during this past winter of 1937–38. So far, no outdoor record has been established for the State of Washington.

I have tried to give as complete a résumé as possible of the occurrence of *Craspedacusta sowerbii* in the United States from 1932 onward, so that it may be used in connection with Dejdar's masterly account. Thus, all known American records may be conveniently referred to.

I believe that the increasing number of records through the years is but a simple function of population increase, rather than

any actual increase in the number of occurrences. The greater the population, the greater the chance that these briefly enduring forms will be seen by some one.

In America, to date, *Craspedacusta sowerbii* has been noted in the District of Columbia, in eighteen[1] different states and in the Panama Canal Zone. The states are: Alabama, Georgia, Illinois, Indiana, Iowa, Kentucky, Michigan, Missouri, New Jersey, New York, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Virginia, Washington (only in aquaria) and West Virginia.

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[1] Since the above was set up, David Causey (1938, *Science,* 88: 13) has recorded two observations establishing the occurrence of *Craspedacusta* in Blue Lake near Prescott, Arkansas, late in the summer of 1927, and in 1937 in a pond near Stamps, in the same state, also late in summer. This increases the total number of states to nineteen.

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WHITING'S HYPOTHESIS AND PTEROMALUS: A CRITIQUE OF DOZORCEVA'S (1936) STUDY[1]

DOZORCEVA (1936) investigated the inheritance of the "sex-linked mutant red-eye color" in *Pteromalus puparum*, in relation to the theory of sex-determination elaborated by P. W. Whiting for *Habrobracon*, and concluded that the theory accommodates her facts.

In view of the significance of work of this sort for an understanding of the mechanism of sex-determination in arrenotokously parthenogenetic forms a close examination of her work seems desirable.

Mutant red-eyed males (the red-eye color being recessive to the normal dark) were mated to normal females, and some of the F_1 females were backcrossed, while others were allowed to lay parthenogenetically. In the latter case males only were obtained and these consisted of red-eyed and dark-eyed forms in equal numbers. In the back-crossing experiments three categories of results were obtained: (1) "Either only normal individuals were produced" or (2) "normal males and an insignificant number of red-eyed females" or (3) "*vice versa*" (presumably meaning "red-eyed males and an insignificant number of normal females"). Dozorceva supplies a table of figures in addition to this general statement, but although it will be unnecessary to deal with it in detail here it can readily be shown to be inconsistent with the demands of Whiting's theory.

To facilitate discussion it is desirable to recount the postulates involved in Whiting's theory. They are: (1) The females are heterogametic (XY); (2) males of two sorts, (X) and (Y), re-

[1] Read at the Edinburgh Meeting of the Genetical Society, June, 1937.

sult from parthenogenetic reproduction; (3) fertilization is differential and selective in that an egg can be fertilized only by a sperm bearing a sex-chromosome of a kind contrasted to that borne by the egg itself; (4) only rarely does fertilization result in diploid homogamety, and the individuals so produced are males.

The results of the parthenogenetic laying by the F_1 females is claimed by Dozorceva to be in accord with Whiting's hypothesis, and while this is true it is no less true that the same result would be obtained whether the females were hetero- or homogametic if the factors for eye-color happened to be autosomal.

According to the theory we should expect two categories of results in the backcrosses according to the nature of the relationship existing between the factor for eye-color and the sex-chromosomes in the females. On the one hand, if the factor for normal eye-color is associated with a sex-chromosome of the same kind as that carried by the inseminating male there would be, in the absence of crossing-over: (1) Red-eyed females from fertilized eggs carrying the recessive factor; (2) red-eyed males from unfertilized eggs carrying the recessive factor; (3) normal-eyed males from eggs unfertilizable by the sperms concerned; and this class should be as numerous as (1) and (2) together.

Crossing-over would result in the addition of another class, that of normal females, and a shift in the numbers of normal and red-eyed males according to the degree of crossing-over.

On the other hand, if the factor for normal eye-color is associated with a sex-chromosome of the opposite kind to that carried by the inseminating male we should expect, in the absence of crossing-over: (1) Normal females from fertilized eggs bearing the normal factor; (2) normal males from unfertilized eggs carrying the factor for normal; (3) red-eyed males from unfertilizable eggs; and this class should be as numerous as (1) and (2) together.

Crossing-over would mean the addition of another class, that of red-eyed females, and corresponding alteration in the relative numbers of the kinds of males.

If we now compare these expected results with what she obtained and if we make every allowance for a 7.63 per cent crossover in eight of the ten backcrosses made and for 28.27 per cent in the others it will be seen that it is impossible to derive any of her three categories of results if the genetics of sexuality and

eye-color in *Pteromalus puparum* proceed according to the theory of P. W. Whiting.

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THE FAILURE OF HOST GENOTYPE TO AFFECT CROSSING-OVER IN AN IMPLANTED OVARY IN DROSOPHILA MELANOGASTER

IT has been shown by Schultz (Morgan, Bridges and Schultz 1932, 1933 and 1935) and confirmed by several other investigators that in *Drosophila melanogaster* and *D. pseudoobscura* (MacKnight 1937) the presence of an heterozygous inversion in one pair of chromosomes causes an increase in crossing-over in the other chromosome pairs. Schultz (1933) has attempted to explain this effect on a purely mechanical basis. Schultz's explanation, however, does not explain at least two known facts: (*a*) a long inversion has no effect on crossing-over in the chromosome pair which bears it. For example, the per cent. of double crossing-over obtained by Siderow, Sokolow and Trofimow (1936) for the yellow-forked interval from females heterozygous for the sc^9 inversion is 5.09, that obtained by Steinberg (1936) for the same interval from females whose chromosomes were normal is 4.6; (*b*) the increase in crossing over per unit map length caused by heterozygous inversions in other chromosomes is directly proportional to the metaphase length of the affected chromosome (Steinberg 1937). The present authors felt, therefore, that the explanation of this phenomenon had to be sought in another direction. In view of the marked physiological effects which most, if not all, inversions cause in the flies that bear them it was thought that possibly some physiological change was responsible for the interchromosomal effects of inversions on crossing over. The experiment reported below is an attempt to test an hypothesis of this sort.

Ephrussi and Beadle (1935) showed that transplanted ovaries may become attached to the host oviducts and that offspring may be recovered from the implanted ovaries. The same authors Beadle and Ephrussi 1937) showed that crossing-over may occur

in the implanted ovaries. Since it is known that the Curly and Payne inversions when combined cause a 285 per cent. increase in crossing-over between yellow and echinus (Steinberg 1936), it was decided to implant ovaries from mature female larvae heterozygous for yellow and echinus ($\frac{y}{ec}$) into mature female larvae heterozygous for these two inversions

$$\left(\frac{Cy\ C_2L \cdot C_2R\ cy}{Pm};\ \frac{C_3\ LP\ Dfd\ C_3\ RP\ ca}{H}\right).$$

When the host females eclosed they were mated to *y ec* males. Crossing-over was followed in the offspring which were known to have arisen from eggs derived from the implanted ovaries. Controls consisted of a backcross of $\frac{y}{ec}$ females to *y ec* males. Table 1 shows a summary of the data.

TABLE 1

SUMMARY OF THE DATA OBTAINED FROM THE FOLLOWING 2 CROSSES: (a) $\frac{y}{ec}$ ♀♀ X *y ec* ♂♂ (CONTROLS); (b) $\frac{Cy\ C_2L \cdot C_2R\ cy}{Pm};\ \frac{C_3\ LP\ Dfd\ C_3\ RP\ ca}{H}$ ♀♀ WITH IMPLANTED $\frac{y}{ec}$ OVARIES X *y ec* ♂♂ (Tests)

	♀♀				♂♂					
	y	*ec*	*y ec*	*++*	*y*	*ec*	*y ec*	*++*	Per cent. cross-over	N
Controls	1,229	1,215	36	44	1,239	1,236	59	30	3.3	5,088
Tests ..	90	87	1	4	106	105	6	4	3.7	403

It is clear that no effect of the inversions present in the host was manifested upon the crossing-over shown by the implanted ovary. (The difference between the control and test crossover values is 0.4 ± 0.3). Although the data indicate that crossing-over is a function of the genotype of the ovary in which it occurs (at least when ovaries of mature larvae are transplanted) it does not eliminate the possibility of such an effect as we have postulated; it merely indicates that if there is such an effect other methods than those employed here must be used.

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THE GENUS AND SPECIES IN RELATION TO EVOLUTION AND TO SYSTEM

THE problem of a definitive and uniform relation between species and genus stirs into life all the age-old problems of analysis. The concept of nature inherited from earlier thought was eminently classificatory. The model of medieval science was a neat symmetrical hierarchy, something on the pattern of a dove-cote, with at the apex a single pigeon-hole, in the next lower row two pigeon-holes, below this four pigeon-holes, and so forth. This symmetrical scheme is an unconscious background or motivation of later thought. The dichotomous arrangement is not insisted upon; but classification is expected to approach it, and the division of one genus into a hundred species while another divides only into two is felt to be anomalous. Especially insistent is the expectation that the classes of things will arrange themselves into a series of definite ranks or levels as we proceed from individual specimens to a most inclusive class. In this case we ought to be able to define the species, the genus, the family and phylum, etc., in unambiguous terms. This conception of a symmetrical hierarchy is encouraged by experimental science, for example chemistry, which reaches a neat classification of its substances into elements, groups and compounds. There is a conspiracy to overlook the artifactual or unnatural origin of the substances and processes analyzed by experimental science, and the degree to which the formulae of experimental theory define uniformities of experimental procedure rather than uniformities of natural articulation.

It was always realized that the dove-cote classification of things breaks down when we pass from the species to the individual. No one expected, that is to say, an approximately equal number of occupants in the different species. The members of one species

might be countable, those of another species innumerable. But why, in this case, should we not expect one genus to possess only a few species and another a great many. There is a sense in which every species is an individual entity, covering just such an area of space, enduring through some particular interval of time, and portraying a unique history. A species is a natural unity, with interdependent parts and conserved in its existence and character by interbreeding, pervasive geographical climate and other conditions. What *natural* principle could set a maximum or a minimum to the number of species bearing the same generic character?

In truth, of course, the whole conception of a dove-coted or neatly pigeon-holed nature is to-day obsolete. It is a scholastic conception proper to the Middle Ages and invalidated by a dynamic science. In its place we have the concept of a nature in evolution, a nature which proceeds always in individual units, these units being subject to change. There can be no question of a system of nature, nor of a single, completed and definitive classification. We see that the old dichotomous classification dimly envisaged the truth that new forms appear as differentiations of earlier forms; but this is only to recognize, in one of its many aspects, the radical continuity of nature. Classification assumes, on the contrary, a radical discontinuity of nature, moderated by the appearance of an inexplicable likeness among the discontinuous parts. Evolutionary science, contrasted with merely systematic science, thus moves to radically opposite concepts of fact and modes of analysis. It sees in nature an articulate complex of spatio-temporal flow, analogous to a fibrous tissue the particular fibers of which may divide, cross and even re-integrate in unpredictable and innumerably diverse ways. In face of a world of this sort, classification becomes merely the first tentative step to a disentanglement or reconstruction of the whole fibrous tissue, in its historic growth; and while the preliminary classification implements this historical reconstruction, it is also subject to revision in the light of its findings.

The specific and generic discontinuities which appear in a non-historical and merely spatial survey give place to spatio-temporal continuities when we turn to an evolutionary survey. It is possible, within the short temporal span of observed fact, to follow the continuous articulation of nature. We see hybridization, the emergence of new form, the extinction of old form, etc. But our reconstruction of the larger past, *i.e.*, the larger movement of nature, is of course dependent upon hypothesis based on present

fact; and this means that a tentative analysis and classification of existent form is the preliminary to an evolutionay understanding of natural movement. But we must never make definitive this preliminary classification, since to do so would be to confine inquiry within arbitrary and casual dogma. This is just what we should be doing if we made definitive the distinction between the genus and the species.

It would seem that if there be a radical discontinuity in nature, proper to the establishment of a definitive classification, that discontinuity exists only at the microscopic level. Certain macroscopic characters may be determined by microscopic genes or other entities. In nature, however, these self-determinate entities, if they exist, exist as factors in natural units and not as natural units themselves. The individual plant, and even the individual cell, exist as organic complexes within which these factors work; and the individual units can not be defined merely in terms of the discontinuous elements. In actuality, accordingly, the discontinuous character of nature remains subordinate to the blended, interdynamic or continuous aspect of nature. Never in a natural science like botany, nor for that matter in a *natural* chemistry, shall we have the precise and definitive typification that we find in experimental chemistry.

There is no reason, however, why we should not prosecute, alongside the natural sciences of life, experimental disciplines analogous to experimental physics and chemistry. The classifications and definitions of such disciplines would hold of experimental and artifactual processes, and could not be directly attributed to natural fact. But they could—or rather we should say they already do—cast light on the processes of nature, and constitute a useful auxiliary to the natural as distinct from the experimental scientist.

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SOMATIC CROSSING-OVER AND SOMATIC TRANSLOCATIONS

AN analysis of mosaic spots on diploid individuals of *Drosophila melanogaster* has led to the conclusion that they are the results of somatic exchanges between homologous chromosomes at homologous loci (Stern, 1936). Recent evidence obtained in the endosperm of *Zea mays* (Jones, 1938), on the other hand, has been thought to demonstrate the occurrence of somatic non-homologous exchanges (translocations) involving random groups of chromo-

somes. This interpretation is based on the finding of twin spots showing phenotypic changes related to genes located in non-homologous chromosomes.

On the basis of the translocation hypothesis, Jones suggests that the mechanism of mosaic production in *Drosophila* is also of the nature of random exchanges. Such an assumption seems to conflict with the fact that no spots resulting from somatic exchanges involving non-homologous chromosomes have been observed. Provided that the frequency of such exchanges is of the same order of magnitude as that of homologous exchanges, ample opportunity for their discovery had been available in experiments in which loci of both the first and third chromosomes had been suitably "marked" (Stern, *l.c.*). However, Jones suggests that in *Drosophila* cells resulting from non-homologous exchanges generally may be so unbalanced due to the possession of duplications and deficiencies that they will not reproduce and thus not give rise to observable spots. According to this view the mosaics actually obtained are regarded as being the rare surviving cases in which random exchanges happened to affect homologous loci.

Although it is true that many unbalanced conditions are not compatible with survival of cells, certain experiments show that at least some such constitutions result in observable mosaic conditions. They were obtained in flies in which the exchanges involved regions heterozygous for an inversion or a ring condition of the chromosome (Stern, *l.c.*, pp. 685–718). Here even homologous exchanges mostly lead to the production of nuclei with unbalanced chromosome sets. The resulting cells produce spots of characteristic and unique appearance. They are restricted to single setae of sub-normal length and diameter in contrast to the spots obtained in flies of regular chromosomal constitution which enclose one to many setae of normal size. The very rare occurrence, if not the complete absence, of abnormal spots in flies of regular constitution is a strong indication that somatic exchanges in *Drosophila* occur prevailingly, if not exclusively, at homologous loci. The term somatic crossing-over for such exchanges seems to be an appropriate one.

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